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DEVELOPMENT OF CHERRY AND PEACH FRUITS AS AFFECTED BY DESTRUCTION OF THE EMBRYO[1]

H. B. TUKEY

(WITH SEVEN FIGURES)

Introduction

That there is a relation between seed formation and fruitfulness is well known, to cite only the work of DETJEN (5) and KRAUS (8). Without fertilization, the peach, cherry, and plum fail to set fruit; and the apple and pear only rarely do so. Moreover it has been shown by many workers, including BRITTAIN and EIDT (1), CRANDALL (2), KOBEL (7), and MURNEEK (14), that the total number of seeds which a fruit contains affects its performance and development. Apple fruits with a high seed content are less likely to absciss prematurely and are most likely to reach the size and shape typical of the variety.

There is, however, little information available upon the relation between fruit development and seed development, as distinct from the relationship between fertilization and set and development of fruit. TUKEY (17, 18) has shown that with the sweet cherry (*Prunus avium* L.) and peach (*P. persica* Stokes) there is a correlation between early ripening of the fruit and embryo abortion. Again it is common observation that insect attack upon developing fruits affects them differently. Fruits of the apple which are attacked severely by

[1] Journal Paper no. 134 of the New York State Agricultural Experiment Station.

aphids will cling to the tree tenaciously, whereas apples which are attacked by the codling moth drop or may ripen prematurely. Some of these insects attack the developing seed while others attack only the flesh.

All of these facts point to a direct relationship between fruit development and seed development. The work here reported was begun to determine the effect of destruction of embryos and seeds upon the development of growing fruits.

Development of embryo, seed, and pericarp of cherry and peach

It has been shown by CONNORS (3), HARROLD (6), LILLELAND (9), and TUKEY (18) that the fruits of the peach develop in three clearly defined stages, and by TUKEY (17, 19) that the fruits of the sweet and sour cherry behave similarly. In each there is a period of rapid development of the pericarp beginning about the time of full bloom (stage I), a period of retarded pericarp development during mid-season (stage II), and a period of rapid pericarp development just prior to fruit ripening, often referred to as the "final swell" (stage III).

The length of these periods varies with the variety, the species, and the season. The period of first rapid development of the pericarp (stage I) following full bloom is the most uniform. Its duration is nearly identical for the varieties in a given class of fruit (18, 19). That is, at Geneva, New York, stage I has a duration of seventeen days for the sour cherry (*Prunus cerasus*) regardless of the variation in time of fruit ripening of the different varieties. For the sweet cherry (*P. avium*) stage I continues for twenty-one days, and for the peach (*P. persica*) for forty-nine days (18). During this period the nucellus and integuments attain maximum size.

Stage II (period of retarded increase of the pericarp) is correlated directly with the season of fruit ripening. Early-ripening varieties have a short period of retarded development whereas late-ripening varieties have a longer period. In the case of the sour cherry, for example, the duration is five days for an early-ripening variety which attains full maturity forty-one days after full bloom; twelve days for a mid-season variety, ripening fifty-seven days after full bloom; and

twenty-eight days for a late variety, ripening sixty-six days after full bloom. At the beginning of this period the stony pericarp begins to harden.

Stage III (period of second rapid increase of pericarp) is variable and continues until final ripening.

The development of the embryo also progresses in three distinct stages, but not necessarily parallel with those of the pericarp (**17, 18, 19**). Following full bloom, while the pericarp and nucellus and integuments are in stage I, the embryo is in a state of arrested development which terminates abruptly in rapid increase, at just the time that stage II of development of the pericarp commences. The initiation of rapid increase of the embryo is correlated also with the initiation of hardening of the stony pericarp and with attainment of maximum size by the nucellus and integuments.

In late-ripening varieties of stone fruits, the rapid development of the embryo continues to maximum size during the period of retarded development of the pericarp (stage II). Seed formation proceeds to maturity, and late-ripening varieties of stone fruits are characterized by viable seed. On the other hand, in the case of early-ripening varieties, stage III of development of the pericarp begins before the embryo is full size, and such varieties are characterized by many abortive or non-viable embryos.

This situation raises several questions. Does the short duration of stage II in early-ripening varieties result in competition between the embryo and the pericarp and ultimate abortion of the embryo? Does the abortion of the embryo permit the initiation of stage III of development of the pericarp? If the embryo of a peach or cherry were destroyed at stage II of development of the pericarp, would the effect be to cause initiation of stage III and thus make a late-ripening variety ripen as an early variety? The material which follows attempts to answer these and related questions.

Materials and methods

Three varieties of sour cherry and six varieties of peach were used. The sour cherry varieties were Early Richmond, an early-ripening variety; Montmorency, a mid-season variety; and English Morello, a late-ripening variety. The peach varieties were Greensboro, Tri-

umph, Arp, and Lola, early-ripening varieties; Elberta, a late mid-season variety; and Chili, a late-ripening variety. The studies were conducted during the growing seasons of 1934 and 1935 in the varietal orchards of the New York State Agricultural Experiment Station at Geneva, New York.

Growth curves of these varieties have been established during several seasons at Geneva, New York (**17, 18, 19**). The growing season is such that most of the individual fruits on a given tree develop nearly identically. This is in part due to the fact that the blooming period is short, often only 36 to 48 hours, so that the fruits on a tree are fertilized and begin development at approximately the same time. This fact makes it possible to use many specimens for measurements and studies with small error from random sampling. In all this work several specimens have been used for each series of tests, involving between 500 and 600 sour cherry fruits and 300 and 400 peach fruits upon which individual growth measurements have been made periodically.

Growth of the pericarp has been recorded by measuring the length, suture diameter, and cheek diameter, and computing the mean. This figure has been shown to follow volume very closely (**19**). The growth in length of embryo, nucellus, and integuments has been recorded. Although figures are also available for both transverse diameters, they have not been used because length alone has seemed to give the desired information.

For establishing growth curves, measurements have been made on Monday, Wednesday, and Friday of each week from full bloom to fruit ripening, random samples being taken from the trees and critically measured and dissected in the laboratory (**18, 19**). In addition, individual fruits have been tagged on the trees and measured regularly throughout the season.

DESTRUCTION OF EMBRYO.—In order to destroy the seed and the embryos in developing fruits, both a needle and a small hand drill were used. The needle was satisfactory for reaching the seed through the distal end of fruits with the split pits characteristic of some varieties, as the Greensboro peach, and more or less common in other varieties depending upon the season (**16**). For most specimens, however, it was necessary to drill a tiny hole through the fleshy and stony

pericarp into the ovarian cavity, through which a needle could be jabbed or other treatment introduced.

A hand drill of the type shown in figure 1, with a drill having a diameter of 0.038 inch, was found satisfactory. From 200 to 300 drillings could be made in an hour by one individual.

To avoid bacterial or fungous contamination the drills were sterilized and the fruit washed with calcium hypochlorite (2 per cent chlorine) solution. In some instances a similar solution was introduced into the ovarian cavity, in others melted paraffin was used to cover and protect the wound, but the results showed no differences.

FIG. 1.—Hand drill (diameter 0.038 inch) used in destroying embryos

The drill method was finally adopted as standard technique, without sterilization and without wound protection. In most cases the fruit developed a wound callose.

Five different positions of drilling were used, only one position being used for any one fruit, namely, directed at (*A*) the embryo through the distal end of the fruit, (*B*) the hilum through the ventral suture, (*C*) the embryo through the cheek of the fruit, (*D*) the fleshy pericarp without injuring the stony pericarp and without entering the ovarian cavity, and (*E*) the stony pericarp but without entering the ovarian cavity (fig. 2).

Since the seed of the peach and cherry is anatropous with the embryo near the distal end of the fruit, drilling at position *A* strikes the embryo directly. Drilling at position *B* was seldom successful in severing the seed at the hilum, but accomplished the desired result by striking into the seed and the embryo. Drilling at position *C* was in most cases equally as effective as at positions *A* and *B*, by interfering with the seed and often striking the embryo. The most

satisfactory and most easily executed drilling to affect the embryo, however, was in position *A*. Drillings in positions *D* and *E* were made as checks to see what effect injury to the stony and fleshy pericarps incidental to destruction of the embryo might have upon fruit development.

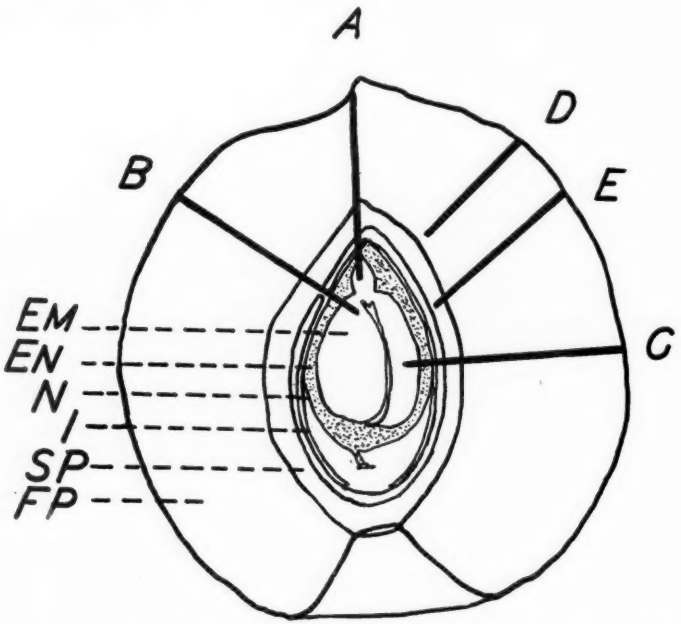

FIG. 2.—Diagram of developing peach fruit showing five positions used in drilling fruit and in destroying the partially developed embryos (*em*, embryo; *en*, endosperm; *n*, nucellus; *i*, integuments; *sp*, stony pericarp; *fp*, fleshy pericarp). Position *A*, through distal end of fruit into embryo; *B*, through ventral suture and hilum into embryo; *C*, through cheek of fruit into embryo; *D*, into fleshy pericarp without injuring seed; *E*, into stony pericarp without injuring seed.

Results

CHERRIES

Cherries were drilled June 5, 1934, in positions *A*, *B*, *C*, *D*, and *E* at the stages of development shown in table I, only one position of drilling being used for any one fruit.

Three days after drilling in positions *A*, *B*, and *C*, fruits of Early Richmond were turning yellow; six days after drilling they were coloring and softening, compared with the characteristic ripening date for the variety two days later. They did not, however, develop high cherry-red color. On the other hand, fruits of both Montmorency and English Morello were yellowing and shriveling three days after destruction of the embryo and were abscissing three days later. Examination of the seeds three days after drilling showed them browning and shriveling, and six days after drilling both the embryo and the entire seeds were disintegrating.

TABLE I

STAGE OF DEVELOPMENT IN DRILLED CHERRY FRUITS OF PERICARP, NUCELLUS AND INTEGUMENTS, AND EMBRYO (JUNE 5, 1934)

VARIETY	MEAN DIAMETER OF FRUIT (MM.)	CONDITION OF STONY PERICARP	LENGTH* OF NUCELLUS AND INTEGUMENTS (MM.)	LENGTH OF EMBRYO (MM.)	STAGE OF FRUIT DEVELOPMENT
Early Richmond.....	10.4	Hard	6.1	3.8 (abortive)	III
Montmorency.....	11.5	Hard	6.3	3.5	II
English Morello.....	12.1	Hard	7.2	3.3 (developing)	II

* Maximum length for all varieties.

Fruit drilled in positions *D* and *E*, so as to injure the fleshy pericarp alone and the fleshy and stony pericarps together but without affecting the seed, grew normally and ripened at the usual time for the respective varieties. In other words, where destruction of the embryos of Early Richmond fruits just entering stage III resulted in earlier ripening, destruction of the embryos of Montmorency and English Morello on the same day, but in stage II, resulted in an abrupt check of fruit development and abscission.

PEACHES

Inasmuch as the destruction of the embryo of cherries indicated a difference in response between early-ripening and late-ripening varieties, this factor was more carefully considered in the tests with peaches. Peaches were drilled variously in positions *A*, *B*, *C*, *D*, and

E at the stages of development and on the dates shown in table II, only one position of drilling being used for any one fruit.

This plan included varieties ripening at different dates from early to very late and all treated similarly on the same date, and also included two varieties of long-growing season, treated similarly at dif-

TABLE II

DATES AND STAGES OF DEVELOPMENT IN DRILLED PEACH FRUITS OF PERICARP, NUCELLUS AND INTEGUMENTS, AND EMBRYO

VARIETY	DATE	MEAN DIAMETER OF FRUIT (MM.)	CONDITION OF STONY PERICARP	LENGTH* OF NUCELLUS AND INTEGUMENTS (MM.)	LENGTH OF EMBRYO (MM.)	STAGE OF FRUIT DEVELOPMENT
Triumph.....	July 26	49.2	Hard	16.5	9.5 (developing)	III
Greensboro...	July 26	56.3	Hard	21.0	12.5 (developing)	III
Arp.....	July 26	35.8	Hard	18.5	11.5 (developing)	III
Lola.....	July 26	47.6	Hard	.....	.....	III
Elberta (I)....	July 26	41.4	Hard	22.0	11.0 (developing)	II
Elberta (II)...	Aug. 14	45.2	Hard	22.0	12.5 (developing)	III
Elberta (III)..	Aug. 17	46.2	Hard	22.0	20.7 (full size)	III
Elberta (IV)..	Aug. 22	47.5	Hard	22.0	20.7 (full size)	III
Elberta (V)...	Aug. 29	49.6	Hard	22.0	20.7 (full size)	III
Chili (I).....	July 26	35.3	Hard	16.3	13.5 (developing)	II
Chili (II).....	Aug. 14	36.5	Hard	16.3	13.8 (developing)	II–III
Chili (III)....	Aug. 17	37.0	Hard	16.3	15.4 (full size)	II–III
Chili (IV).....	Sept. 12	46.5	Hard	16.3	15.4 (full size)	III

* Full size for all varieties.

ferent dates. The early-ripening varieties were all in stage III when treated, while the Elberta and Chili varieties were in different points in stages II and III. The embryos were all in the period of rapid increase when the first drillings were done. In later drillings some of the embryos had approached full size and maturity. In all cases, even with the earliest drillings, nucellus and integuments had reached maximum size and the stony pericarp had begun to harden.

DESTRUCTION OF EMBRYO OF EARLY-RIPENING VARIETIES.—The performance of peach fruits following destruction of the embryo and wounding of the pericarp was similar to that of the sour cherry. In the case of such early-ripening varieties as Triumph, Greensboro, Arp, and Lola, destruction of the embryo resulted in earlier ripening of the fruits. In table III are given the data for the Triumph peach, which may be taken as representative of these four varieties, and with which the other varieties may be compared. Greensboro fruits with embryos destroyed July 26, ripened August 9, five days earlier

TABLE III

FRUIT DEVELOPMENT OF TRIUMPH PEACH AS AFFECTED BY KILLING THE EMBRYO AT DIFFERENT STAGES OF DEVELOPMENT (BLOOMED MAY 22; FRUIT RIPE, AUGUST 12, 1935)

TREATMENT	SIZE OF FRUIT (MEAN OF LENGTH, CHEEK DIAMETER, AND SUTURE DIAMETER) (MM.)		
	JULY 26	JULY 30	AUGUST 9
Embryo killed July 26			
Position A	49.2	53.22	66.45 (soft ripe)
Position C	49.2	53.10	66.35 (soft ripe)
Split-pit jabbed with needle	49.2	54.83	..... (soft, dropped)
Check (pericarp drilled in cheek three times, but embryo not injured)			
Position D	49.2	52.62	63.42 (hard ripe)
Check ("normal" fruit development)	49.2	52.36	64.25 (hard ripe)

than untreated fruits; and Arp fruits with embryos destroyed, ripened August 14, eight days earlier than untreated fruits. Lola fruits with embryos destroyed August 17 ripened August 26, or three days earlier than untreated fruits.

Not only were the fruits of early varieties hastened in fruit ripening by destruction of the embryo, but also the growth rates were increased so that the fruits were larger on the same date than untreated fruits (fig. 3). Furthermore, fruits with embryos destroyed were larger in size *at maturity* than normal fruits *at maturity*, although the increase in size is not large (fig. 4). Fruits of Triumph with embryos destroyed attained at maturity a mean diameter 2 to 3 mm. greater than that of fruits not so treated, fruits of Greensboro

2 to 2.6 mm. greater, of Arp 5.74 mm. greater, and of Lola only 0.42 mm. greater.

DESTRUCTION OF EMBRYO OF LATE-RIPENING VARIETIES.—Destruction of the embryos of late-ripening varieties (Elberta and Chili) on the same day resulted in abrupt check in fruit development, shriveling, and abscission (figs. 5, 6, 7). The effect of embryo destruction was sharp, no measurable increase of fruit size occurring

FIG. 3.—Fruit development of early-ripening variety (Triumph) following destruction of embryo during stage III of fruit development. Embryos destroyed by drilling July 26; fruits photographed August 10. Upper row not drilled; fruits developing typically and not ripe at this date. Lower row drilled; fruits larger, and ripe on same date. (Note drill holes in apex of lower row.)

thereafter (tables IV, V). It must be remembered, of course, that while the embryos of all varieties were in similar stages of development, yet the destruction of embryos of early-ripening varieties occurred in stage III whereas destruction of the embryos of late-ripening varieties on the same day occurred in stage II.

The results from destruction of the embryos of late-ripening varieties at points later along the curve of development differ still further in some respects from those of early-ripening varieties, whereas in other respects they agree. When embryos of Elberta were de-

stroyed August 14, 17, 22, and 29, the fruit was in stage III, and the result was to hasten maturity, just as in the case of the early-ripening varieties, whose embryos were destroyed in stage III. Furthermore, the nearer to the normal ripening date for the variety, the more abrupt was the increase in rate of growth from that of untreated

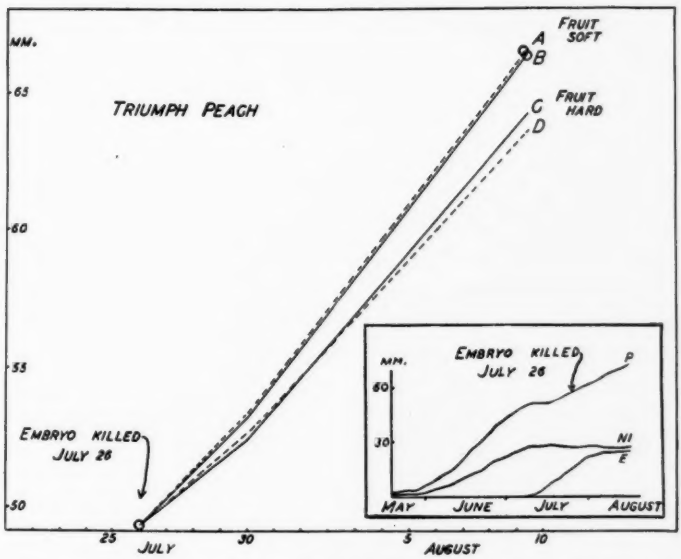

FIG. 4.—Growth of typical fruits of early-ripening variety of peach (Triumph), and of fruits with embryos destroyed by drilling July 26 at point in development indicated in insert (*p*, pericarp; *ni*, nucellus and integuments; *e*, embryo). Position *A*, embryo killed by drilling through distal end; *B*, embryo killed by drilling through cheek of fruit; *C*, pericarp wounded but embryo not injured; *D*, check.

fruit. Destruction at points early in stage III failed to increase the rate of growth although it did hasten ripening. Destruction at points later in stage III resulted not only in larger size at the time of ripening, as compared with untreated fruit on the same date, but also in what appears to be an actually larger size as compared with untreated fruit *at maturity* (fig. 6).

A further illustration of the more rapid increase in the rate of growth as the destruction of the embryo approaches fruit maturity

TABLE IV

FRUIT DEVELOPMENT OF ELBERTA PEACH AS AFFECTED BY KILLING THE EMBRYO AT DIFFERENT STAGES OF DEVELOPMENT (FULL BLOOM MAY 20; FRUIT RIPE, SEPTEMBER 25, 1935)

TREATMENT	SIZE OF FRUIT (MEAN OF LENGTH, CHEEK DIAMETER, AND SUTURE DIAMETER) (MM.)								
	JULY 26	JULY 30	AUG. 9	AUG. 14	AUG. 17	AUG. 22	AUG. 29	SEPT. 12	SEPT. 25
I. Embryo killed July 26									
Position A	41.4	41.3 (shriveling)	30.3 (dropped)	.....	.....	.....	.....	.....	.....
Position B	41.4	42.6 (shriveling)	.... (dropped)	.....	.....	.....	.....	.....	.....
Position C	41.4	40.8 (shriveled)	.... (dropped)	.....	.....	.....	.....	.....	.....
II. Embryo killed August 14									
Position A	.....	.....	.....	45.2	46.0	47.9	53.1	62.2 (softening)	.....
III. Embryo killed August 17									
Position A	.....	.....	.....	.....	46.2	47.5	51.1	62.8 (coloring, softening)	.....
IV. Embryo killed August 22									
Position A	.....	.....	.....	.....	.....	47.5	51.5	63.9 (softening)	.....
V. Embryo killed August 29									
Position A	.....	.....	.....	.....	.....	.....	49.6	62.7 (softening)	.....
Check ("normal" fruit development)	41.4	41.8	43.8	45.2	46.2	47.5	49.6	60.8	60.8 (ripe)
Check (pericarp drilled to pit but embryo not injured)									
Position E	41.4	41.9	44.3	44.6	45.7	47.0	50.0	60.9	60.9 (ripe)
Check (pericarp stung by insect but embryo not injured)									
Position D	41.4	41.4	43.8	45.5	45.6	47.4	50.0	61.1	61.1 (ripe)

TABLE V

FRUIT DEVELOPMENT OF CHILI PEACH AS AFFECTED BY KILLING THE EMBRYO AT DIFFERENT STAGES OF DEVELOPMENT (FULL BLOOM MAY 21; FRUIT RIPE, OCTOBER 4, 1935)

TREATMENT	SIZE OF FRUIT (MEAN OF LENGTH, CHEEK DIAMETER, AND SUTURE DIAMETER) (MM.)									
	JULY 26	JULY 3	AUG. 9	AUG. 14	AUG. 17	AUG. 22	AUG. 29	SEPT. 12	SEPT. 25	OCT. 4
I. Embryo killed July 26 Position A	35.3	35.6 (shriveled)	29.8 (dropped)	.....	.....	.....	.....	.....	.....	.....
II. Embryo killed August 14 Position A	.....	.....	.....	36.5	36.7	37.7	40.0	46.4 (dropped)	.....	.....
Check ("normal" fruit development on same branch)	.....	.....	.....	36.5	36.8	37.3	38.3	44.0	45.4	45.4
III. Embryo killed August 17 Position A	.....	.....	.....	.....	37.0	37.8	40.2	44.0 (dropped)	.....	.....
IV. Embryo killed September 12 Position A	.....	.....	.....	.....	.....	.....	.....	46.5	51.3 (ripe, dropped)	.....
Check ("normal" fruit development on same branch)	.....	.....	.....	.....	.....	.....	.....	46.5	51.1	51.1
Check ("normal" fruit development)	35.3	35.3	35.6	36.5	37.0	37.6	39.8	46.5	49.3	49.3 (softening)

is seen from table IV of the Elberta peach. For example, fruits with embryos destroyed on August 14, 17, 22, and 29 all reached the stage of "soft ripe" on the same date, September 12. The fruits drilled on August 14, however, reached this stage by an increase between August 29 and September 12 of only 9.08 mm. in mean diameter, whereas the fruits drilled August 17 increased 11.67 between these dates, those drilled August 22 increased 12.45, and those drilled August 29 increased 13.15 mm.

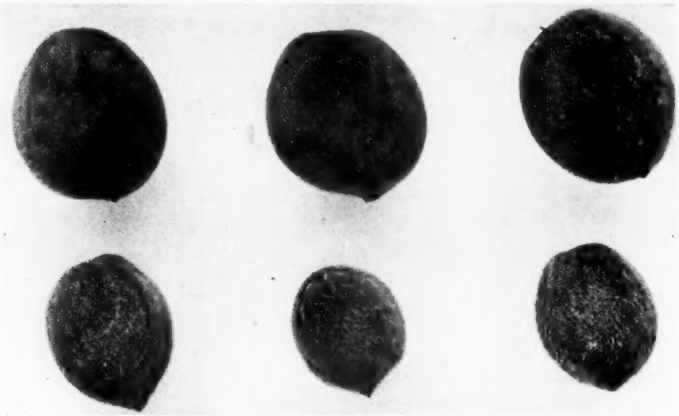

FIG. 5.—Fruit development stopped by destruction of embryo of late-ripening variety (Chili) during stage II of fruit development. Fruits drilled July 26; photographed August 10. Upper row not drilled, fruits developing typically. Lower row drilled, fruits shriveled and abscissed on same date.

The results with the other late-ripening variety, Chili, also differ from those of the other varieties in some respects and agree in others (table V). They agree with Elberta in that the destruction of the embryo in stage II resulted in immediate check in fruit development, with subsequent shriveling and abscission (fig. 5). They agree with all varieties in that the destruction of the embryo late in stage III resulted in an increase in the growth rate and a hastening of ripening (fig. 7).

Most significant, however, are the points of embryo destruction at stages midway between early and late development. It will be re-

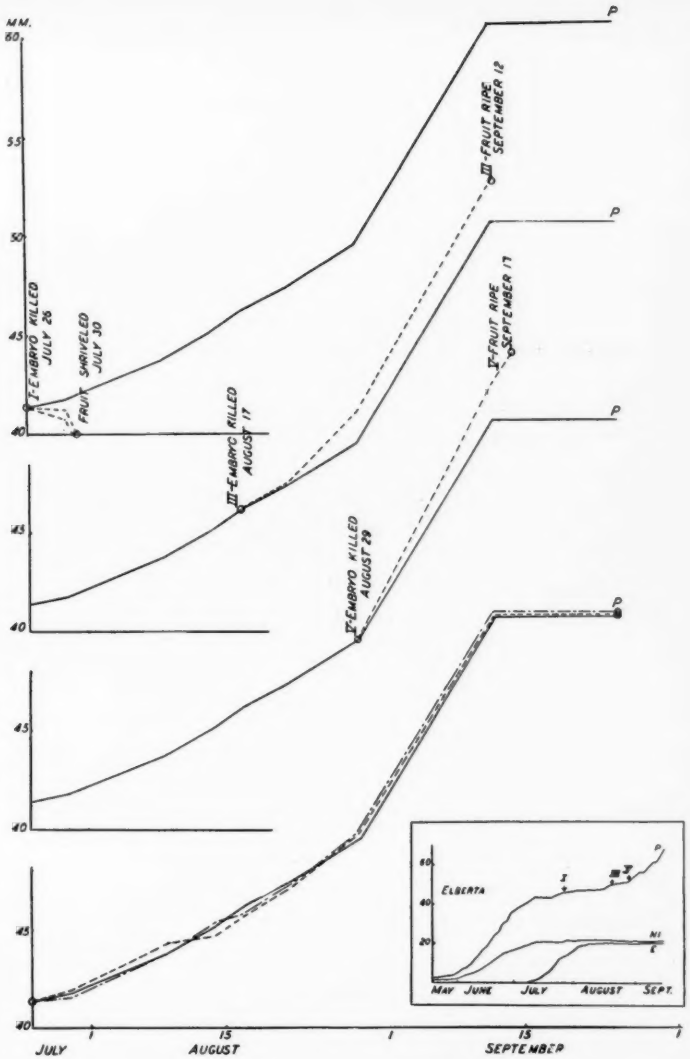

FIG. 6.—Growth of typical fruits of late mid-season variety of peach (Elberta) and of fruits with embryos destroyed by drilling at points in development indicated in insert (*p*, pericarp; *ni*, nucellus and integuments; *e*, embryo).

Solid lines = normal growth curve.

Dotted lines I, III, V = development following destruction of embryos at dates indicated.

Dotted lines in bottom curve = pericarp wounded by insect.

Broken line in bottom curve = pericarp wounded by drilling.

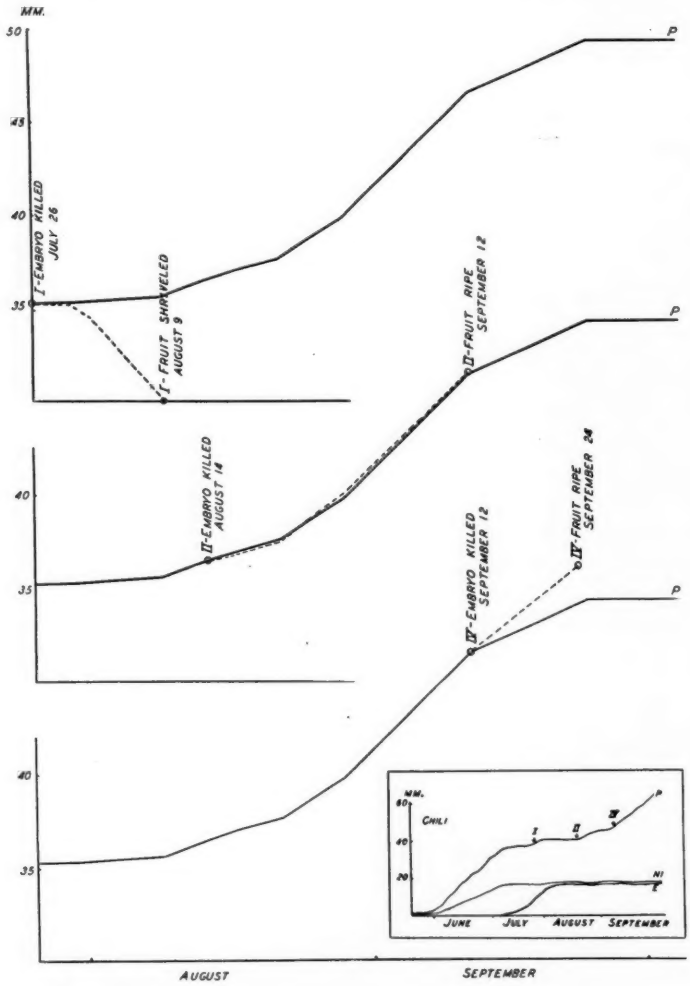

FIG. 7.—Growth of typical fruits of late-ripening variety of peach (Chili) and of fruits with embryos destroyed by drilling at points in development indicated in insert (*p*, pericarp; *ni*, nucellus and integuments; *e*, embryo).

Solid lines = normal growth curve.

Dotted lines I, II, IV = development following destruction of embryos at dates indicated.

membered that stage II of Chili is of relatively long duration. Apparently the destruction of the embryos on August 14 and 17 was at a point in development of the pericarp in transition between stages II and III, because while fruits with destroyed embryos increased in size after treatment, yet such fruits failed to reach full size for the variety and dropped prematurely. Accordingly they were unlike fruits whose embryos had been destroyed at earlier dates, in that they followed the growth curve of untreated fruits for a period whereas the others were checked abruptly in development and later shriveled and abscissed. Also they were unlike fruits whose embryos were destroyed at dates near to maturity of untreated fruits, in that they failed to increase at a rate faster than the untreated fruits although they ripened earlier. Likewise they did not reach full size for the variety. They more nearly approached the behavior of Early Richmond fruits whose embryos were destroyed early in stage III.

WOUNDING OF THE PERICARP.—Quite in contrast to the results from destruction of the embryo were the results from wounding the fleshy pericarp alone and of the stony and fleshy pericarps together but without affecting the seed. Neither the growth rate of the fruit nor the season of fruit ripening was altered from that which is typical of the variety. In fact, in several instances observations were made of the successful and apparently normal development of fruits which were wounded in the flesh by birds and insects. One such instance was carefully measured and followed to maturity, as shown in table IV. The curve of growth and season of ripening were not affected.

In several instances treated and untreated fruits were adjacent on the same branch (table IV). In all such instances the fruits behaved in a manner characteristic of the type of treatment, thus eliminating any possibility of differences in nutritional effect or effect due to position on the tree.

Discussion of data

In most cases, fruits of varieties with different ripening dates did not respond to destruction of the embryo by ripening on the same date. Instead the ripening processes were hastened along the growth curve characteristic of the variety. For example, typical untreated fruits of Greensboro ripened August 14, and those of Arp, August 22.

Destruction of embryos of both varieties on July 26 did not result in fruits ripening on the same day; instead those of Greensboro ripened August 9, and those of Arp, August 12. Again fruits of Lola and of Elberta with embryos destroyed on August 17 ripened on August 29 and September 12 respectively, and fruits of Chili similarly treated failed to reach full size by September 12.

Yet the embryos of Chili were apparently as fully developed morphologically as those of Elberta on the same date, so that it cannot be said that destruction of the embryo is the sole controlling factor in development of the pericarp. Apparently there is a relationship between fruit development and embryo development which is characteristic of the variety, and which is altered by destroying the embryo, but which alone does not control the process of fruit development.

An additional consideration suggested by these data is the effect of such factors as frost and insect attack upon the development of the embryo, affecting in turn the development of the fruit. It might be expected that those agencies which destroy the embryo in early stages of development might cause the fruit to drop, whereas those which affect the fruit without destroying the embryo might not. Again, agencies which destroy the embryo in late stages of development might be expected to increase the growth rate and hasten the ripening of the fruit. General observations confirm these suggestions. Spring frosts which destroy the embryo frequently result in abscission of the fruit. Injury by frost to the pericarp or accessory fruit parts without injuring the embryo, however, does not result in premature abscission, although fruits so injured may display at maturity cracks, russet markings, and other evidences of early season injury. The attacks of the larvae of the codling moth (*Carpocapsa pomenella* L.), which in early season destroy the developing embryo, result in abscission of the fruits. Attacks from the larvae in late season, when the fruits are nearing maturity, hasten ripening of the fruit.

Observations also suggest a relation between pit splitting in peaches and the development of the embryo and the fruit. Among those studying this situation, DAVIS (4) has found a close relationship between the size or growth rate of fruits and the occurrence of

split-pits, in which large fruit sizes are associated with pit splitting. Moreover, he cites MIKI (12) as reporting that the percentage of split-pit is higher in the earlier ripening fruits within the same variety. DAVIS concludes, "These facts would seem to offer additional weight to the suggestion that the occurrence of split-pit is very closely associated with those conditions which cause an increased rate of growth of the fruit."

Subsequently RAGLAND (16), in a morphological study of split-pit, has reported that "embryos of split-pit fruits are aborted in a very high percentage of cases; nevertheless, the flesh develops to maturity as in normal fruits." He has found a group of bundles which he calls funicular bundles, supplying the ovules and anastomosing with the vascular cylinder at the base of the carpel, as distinct from the dorsal bundle lying along but never within the pit and the two ventral bundles lying in the deep grooves of the pit along the ventral suture.

Lignification of the pit does not proceed uniformly but begins along the ventral suture. Since there is little actual union between carpel edges, this region is subject to breakage from stresses set up either by the developing pericarp or by contraction within the pit itself (13, 16). The location of the funicular bundles so near the line of cleavage along the ventral suture renders them especially susceptible to rupturing when the split occurs.

These facts are suggestive of a situation in which splitting of the pit results in checking of the embryo as in early-ripening varieties, or as when the embryo is destroyed by external agencies, as drilling, resulting in turn in a more rapid growth rate of the fruit and earlier maturity.

Other factors, however, may play a part in the results brought about by destruction of the embryo. Among these are the morphological nature of the fruit. In the case of a simple fruit consisting of a single carpel, as in the peach, cherry, and plum, the effect from destruction of the embryo is less easily masked than in the case of multiple or accessory fruits consisting of several carpels and associated stem and receptacle, as the apple and the pear. Destruction of the single developing embryo in a peach might be expected to result in abscission of the fruit, whereas destruction of several or all of the

embryos of an apple might not result in abscission of the fruit, although possibly influencing the development of accessory fruit parts.

Furthermore, the genetic quality of a variety may alter the situation, as suggested by KRAUS (**8**). Thus, the McIntosh apple is self-sterile and self-unfruitful and seldom develops fruits without seed. On the other hand, the Baldwin apple may develop fruits containing only a few seeds, or may even develop fruit parthenocarpically. A further expression of this situation in these varieties is seen in the development of individual carpels following the failure of the embryo. In the case of the McIntosh apple, carpels which do not contain at least one seed are likely to be poorly developed, resulting in angular, lopsided, and otherwise misshapen fruits; while with the Baldwin apple, carpels which contain no developing seed may make growth and development resulting in well shaped fruits typical of the variety. Nutritional factors also play an important part, as when the number of apples which form following fertilization is greater than the tree can develop to maturity. In such case the fruits which drop in the various waves of early-season abscission are likely to be those with low seed count, whereas when the number of apples which form is small in proportion to the total number which the tree is able to develop, many fruits of low seed count may adhere and develop to maturity.

Yet factors which affect the fruit and the embryo equally without injury, such as temperatures, do not disturb the relation between embryo and fruit development. LILLELAND (**10**) in a study of temperature upon fruit development has found that when individual apricot fruits are inclosed in heating chambers, stages I and II of fruit development are accelerated in comparison with adjacent fruits untreated on the same branch. Likewise the stages of development of the embryo are accelerated and in direct relation to the increase in growth of the fruit, so that although treated fruits ripen earlier than untreated fruits, the embryos of treated fruits also develop earlier and are at the stage of development characteristic of the variety at maturity.

General discussion and conclusions

The results presented in this paper have been based largely on morphological observations. Certain physiological features remain.

Among these is the chemistry of "stage of development" as indicated by LOTT (11). That is, although morphologically embryos of Elberta and Chili may seem to be at identical stages of development on a given date, it does not necessarily follow that there may not be some more rapid accumulation or release of materials in one than in the other, or some other biological factor present in one and not in the other, which may be the controlling factor.

MURNEEK (14), in discussing the effects of seed development on the subsequent behavior of flowers, uses the results obtained with *Cleome spinosa* to show that the developing seeds of a plant may be responsible for the reduction in growth of the female reproductive organs, and in this instance bring about sterility. Removal of the fertilized pistils or young seed pods at the proper time resulted in all flowers developing normally and in being fertile. In elaborating upon growth and development as influenced by fruit and seed formation he says, "The question naturally arises as to what may be the means by which the developing embryos are able to draw to themselves and the adjoining tissues most of the available food supply. To accomplish such results a definite and powerful mechanism certainly must be at the disposal of the reproductive organs. At present we are entirely ignorant as to its nature beyond the mere conjecture that it may be either of a general physiological or a specific enzymatic or hormonic character."

The data presented in this paper, while not adding information that might help to answer this question, do support the theory propounded by MURNEEK and others and carry the principle to the point where embryo development is shown not only to influence plant development in general but also markedly to influence fruit development.

In answer to the question raised by TUKEY (17, 18) as to whether embryo abortion is responsible for the accelerated growth of pericarp in early-ripening varieties of cherry and peach, or whether it is the rapid growth of the pericarp which brings about embryo abortion, these results indicate that the embryo is the controlling factor.

Do they mean, however, that the pericarp does not develop in its final rapid growth just preceding maturity until the embryo "releases" it, or until some balance is attained between pericarp and seed parts, or until some substance is developed within the seed

parts which favor development of the pericarp? And do they mean that in the case of early-ripening varieties the release comes earlier so that balance is attained earlier, or a growth promoting substance is developed earlier than in the case of late-ripening varieties? It would seem that there are at least two factors in operation in this situation, the stage of development at which embryo destruction occurs and the characteristics of the variety.

Summary

1. Development of the fruit of the cherry and peach is in three clearly marked stages: stage I, rapid increase of the pericarp from about the time of fertilization to mid-season; stage II, retarded increase during mid-season; and stage III, second rapid increase to fruit maturity.

2. Embryos of three varieties of sour cherry (*Prunus cerasus*) and six varieties of peach (*P. persica*) were destroyed at various stages of development by jabbing through split pits with a needle and by drilling through the pericarp. Fruits which were wounded in the fleshy pericarp and stony pericarp but without injury to the embryo, and fruits which were attacked by insects, were also followed in development.

3. Destruction of the embryo early in stage II of fruit development resulted in abrupt check in fruit development, shriveling, and abscission.

4. Destruction of the embryo in the transition between stages II and III of development of the pericarp resulted in growth of the fruit at a rate similar to that of untreated fruit for a limited period, but ended with earlier ripening and failure to reach full size.

5. Destruction of the embryo in stage III of development of the pericarp resulted in increased growth rate and earlier ripening, and occasionally increased size over that attained by untreated fruits at maturity.

6. Wounding of the fleshy and stony pericarps, without injuring the seed, did not alter the growth rate of the fruit.

7. The nearer the ripening date typical of the variety at which the embryo was destroyed, the greater was the increase in growth rate of the pericarp.

8. Two factors seem involved in the relation between embryo and pericarp: (*a*) the stage of development of the fruit and of the embryo at which the embryo is destroyed, and (*b*) the genetic makeup of the variety controlling this relation.

9. Development of the embryo is shown to bear a definite relation to the development of the fruit quite distinct from the effect of fruit and seed formation upon the entire plant.

10. In early-ripening varieties of cherries and peaches it would seem that abortion of the embryo is the factor which affects pericarp development rather than the reverse.

11. Frost, insect attack, and pit splitting of peaches are discussed in relation to their effect upon the development of the embryo and of the fruit. The morphological nature of fruits is considered in this regard, together with nutritional factors.

NEW YORK STATE AGRICULTURAL
EXPERIMENT STATION
GENEVA, NEW YORK

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CONTROL OF ATMOSPHERIC HUMIDITY IN CULTURE STUDIES

HENRY HOPP

(WITH ELEVEN FIGURES)

The objectives of this paper are threefold: (a) to present a summary of the methods which have been used to control relative humidity; (b) to report several new procedures for their practical application; and (c) to consider their relative value in culture studies. The instruments and new procedures reported in this paper have been developed in studies concerned with the culture of wood-destroying fungi.

Control of the atmospheric humidity within culture chambers can be secured by chemical and physical means.

Chemical control of humidity

Chemical control of the humidity is attained through the evaporation of water from a solution, the vapor pressure of which is regulated by the presence of dissolved chemicals. Various relative humidities may be secured quantitatively through the use of the same solute in different concentrations, or qualitatively by using different kinds of solutes in saturated solutions.

QUANTITATIVE CHEMICAL CONTROL

Any soluble chemical which causes an appreciable reduction in aqueous vapor pressure may be used to obtain quantitative chemical control of the humidity (**13**). Sulphuric acid (**5, 9, 15, 18, 20, 22**) is most generally used. Sodium chloride has also been employed to secure percentages of relative humidity between 75 and 100 (**4**). The relation between relative humidity and concentration of sulphuric acid is shown in figure 1. The relative humidity secured at a definite concentration of acid is approximately constant (**20**) through the usual temperature range of culture incubation (15° to 30° C.), although it is best to maintain a constant temperature. For experi-

ments of short duration, the method can be employed very conveniently in a set-up using petri dishes (fig. 2). The dishes used are of two sizes, the smaller one (which is uncovered) being placed within the larger. The substratum is contained in the inner dish and the solution is poured into the outer one. The presence of sul-

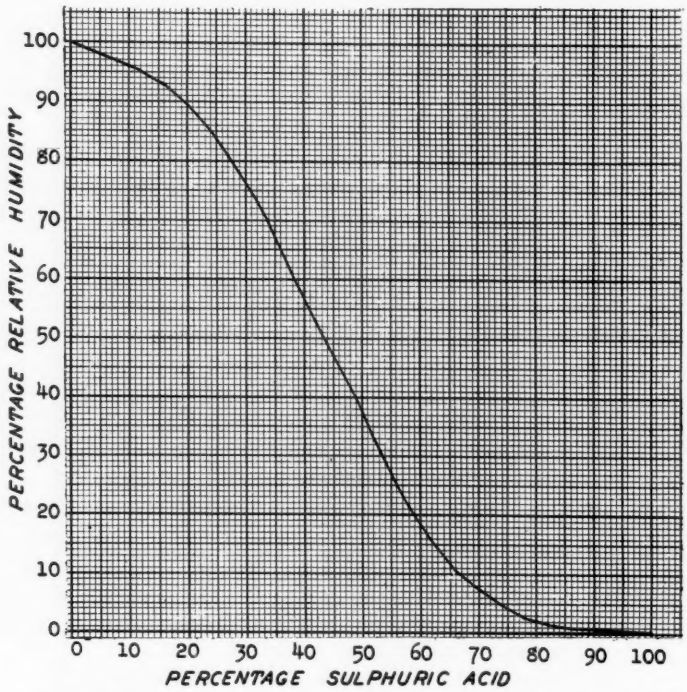

FIG. 1.—Relative humidity of atmosphere obtained above sulphuric acid solutions of various concentrations. Data from STEVENS (15).

phuric acid, the vapor pressure of which is practically nil, has no apparent effect on fungi growing in the inner dish.

The quantitative method of control, although simple and inexpensive, is of limited application. The relative humidity can be regulated effectively only in chambers of small volume, since diffusion from the surface of the solution is slow. If the water exchange between the air and the solution is considerable, the resulting change

in concentration of the solution will affect the relative humidity. A digression of 1 per cent from the original concentration of a sulphuric acid solution causes a variation in relative humidity of 1 to 2 per cent at ordinary temperatures. In order to prevent a significant change in concentration of the solution, therefore, the humidity chambers must be closed to the outside atmosphere. This is a handicap when living organisms are the subject of experimentation, since under certain conditions their respiratory processes cause a considerable change in the chemical composition of the air. The extent of this change was determined by measuring the oxygen consumption of various fungi. It was found, for example, that the

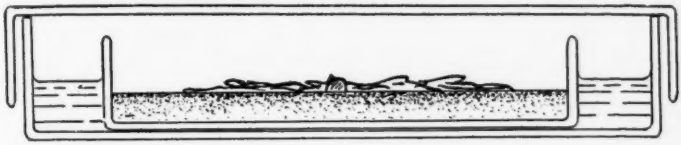

FIG. 2.—Petri dish set-up for culturing organisms under conditions of controlled atmospheric humidity.

oxygen content of the air within a Novy jar of 2.5 liters capacity which contained two petri-dish cultures of *Polyporus versicolor* (L.) Fries was decreased by 5 per cent in 24 hours; and after 5 days the oxygen content was only 0.56 per cent.

Control is ineffective also when large quantities of hygroscopic or hydrated material are present in the chamber which contains the solution. A hygroscopic material, such as dry wood blocks, if placed within chambers operated at a high relative humidity, would absorb considerable atmospheric water. As a result the concentration of the sulphuric acid solution would be significantly increased. Hydrated materials, such as agar media, if placed in chambers operated at a low relative humidity, would cause considerable dilution of the sulphuric acid solution. When small quantities of agar medium are used, however, the dilution of the solution is so slight that the relative humidity is not considerably changed.

QUALITATIVE CHEMICAL CONTROL

Saturated solutions of various chemical substances (table I) may be used to control the humidity of the atmosphere (**5, 6, 1, 7, 19**).

The mechanics of the method are relatively simple. A saturated solution of the substance is made and an excess of the solute is then

TABLE I

APPROXIMATE RELATIVE HUMIDITY OF ATMOSPHERE AT ROOM TEMPERATURE OBTAINED ABOVE SATURATED SOLUTIONS OF VARIOUS SUBSTANCES. DATA FROM SPENCER (13)

SUBSTANCE	PERCENTAGE RELATIVE HUMIDITY
$CaSO_4 \cdot 5H_2O$	98
K_2SO_4	98
$Na_2SO_3 \cdot 7H_2O$	95
$Na_2HPO_4 \cdot 12H_2O$	95
KNO_3	94
$Na_2SO_4 \cdot 10H_2O$	93
K_2HPO_4	92
$Na_2C_4H_4O_6 \cdot 2H_2O$	92
$BaCl_2 \cdot 2H_2O$	88
K_2CrO_4	88
$Na_2CO_3 \cdot 10H_2O$	88
KCl	87
$KHSO_4$	86
KBr	84
Na_2SO_4	82
NH_4Cl	79
$H_2C_2O_4 \cdot 2H_2O$	76
$NaCl$	76
$NaNO_3$	76
$CoCl_2 \cdot 6H_2O$	67
$NaNO_2$	66
NH_4NO_3	64
$NaBr \cdot 2H_2O$	58
$Mg(NO_3)_2 \cdot 6H_2O$	53
$Ca(NO_3)_2 \cdot 4H_2O$	52
$Na_2Cr_2O_7 \cdot 2H_2O$	52
$NaHSO_4 \cdot H_2O$	52
KNO_2	45
$K_2CO_3 \cdot 2H_2O$	43
$Zn(NO_3)_2 \cdot 6H_2O$	42
$MgCl_2 \cdot 6H_2O$	33
$CaCl_2 \cdot 6H_2O$	32
$KC_2H_3O_2$	20
$LiCl \cdot H_2O$	15
$H_3PO_4 \cdot \frac{1}{2}H_2O$	9

added. In this way permanent saturation is obtained, since absorption of atmospheric water into the solution causes dissolution of a portion of the excess solute.

Owing to the slow rate of diffusion from the surface of the solution, effective regulation of the atmospheric humidity can be attained only in chambers of small volume. This disadvantage can be overcome either by using fans in the humidity chamber or by humidifying a current of air, which is then passed through the chamber. Continuous replenishment of the air is especially desirable when rapidly respiring material is the subject of experimentation (6). A method to procure continuous renewal of the air within the chamber is shown in figure 3. The air is bubbled through a saturated

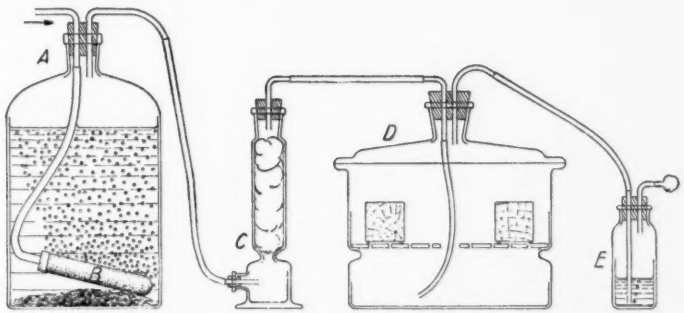

FIG. 3.—Qualitative chemical control of humidity in fresh-air chambers under sterile conditions: *a*, humidifying jar; *b*, diatomaceous filter; *c*, cotton filter; *d*, humidity chamber containing experimental material; *e*, trap-jar.

salt solution, which is contained within a humidifying jar. It is then filtered in a tower packed with absorbent cotton in order to remove suspended droplets of the solution. The conditioned air passes into the humidity chamber and is discharged through a trap-jar containing some of the salt solution. Thorough humidification of the air is assured by dividing it into small bubbles within the humidifying jar. A diatomaceous filter is satisfactory for the purpose. An aquarium aerator consisting of a 1-inch cube block of porous carborundum, into which a piece of glass tubing is cemented, or simply a block of porous-vesseled wood sealed into the end of a rubber tube, can also be used. The humidity chamber is freed of organisms by sterilization with acetic acid, which may be evaporated by operating the apparatus for several days before the start of the experiment.

All contaminants are caught in the salt solution and in the cotton filter.

The apparatus requires little attention once it is adjusted. The velocity of the air stream can be regulated by either a pet-cock or a pressure regulator on a compressed air line. The rate of bubbling in the trap-jar indicates the air velocity. The pressure required in the air line depends on the kind of porous block employed in the humidifying jar. A diatomaceous filter requires a minimum pressure of about 12 pounds per square inch. Five pounds' pressure is needed to force the air through an aquarium aerator. When the humidity of the conditioned air exceeds that of the air supplied by the pressure line, the porous block becomes clogged. This is due to the absorption of water from the saturated solution and the consequent crystallization of solute. To avoid this situation, the air should be humidified before it passes through the porous block. A simple way to saturate the air is to bubble it through a small jar of water.

The writer has found this apparatus entirely dependable in tests conducted over a period of several years. With this set-up the air is not only humidified but is also sterilized. The method is well suited for the control of humidity in studies involving gas exchange (19).

In determining the substance to be used in attaining a desired relative humidity, considerable selection is often possible. Three factors should be considered in making this choice: (1) the solubility of the solutes, (2) the effect of temperature on the change in their solubility, and (3) the influence of temperature changes on the aqueous vapor pressure of their saturated solutions. The effects of these factors are illustrated in the case of the choice between $NaNO_3$ and NaCl, either of which can be used to obtain a relative humidity of 76 per cent at 23° C. (fig. 4). Owing to the difference in solubility of these salts, it is necessary to use about twice as much $NaNO_3$ as NaCl in order to prepare saturated solutions. On the basis of expense and convenience alone, the latter salt would be preferred. The effect of temperature variation on the solubility of the salt is another important factor. If a slight fall in temperature causes a relatively great decrease in solubility, a considerable amount of the salt will be precipitated out of the solution. The physical effect of

this result is important since the solute is precipitated not only on the bottom of the humidifying jar but also on the porous block, which then becomes clogged. On the other hand, if the temperature rises, a considerable amount of the excess salt lying at the bottom of the humidifying jar will be dissolved. During the period of time

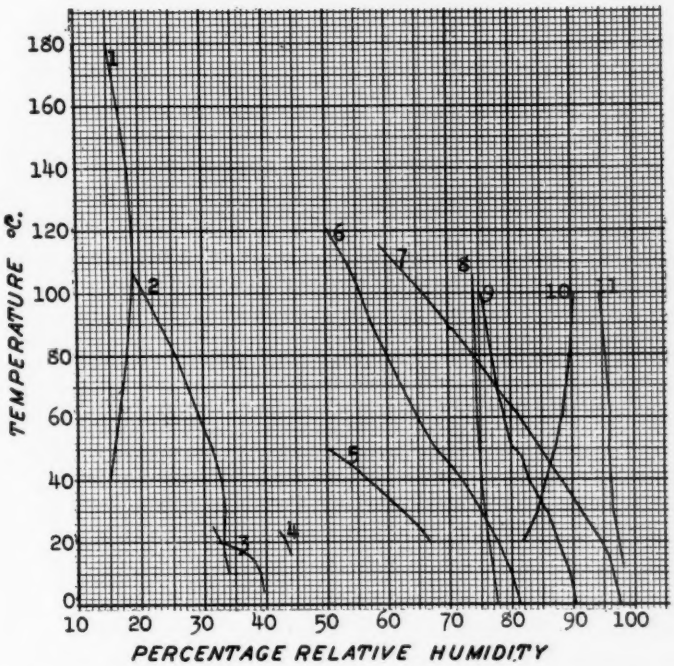

FIG. 4.—Effect of temperature on relative humidity obtained with saturated solutions of various substances: (1) $CaCl_2 \cdot 2H_2O$, (2) $MgCl_2 \cdot 6H_2O$, (3) $CaCl_2 \cdot 6H_2O$, (4) $K_2CO_3 \cdot 2H_2O$, (5) $CoCl_2 \cdot 6H_2O$, (6) $NaNO_3$, (7) KNO_3, (8) NaCl, (9) KCl, (10) Na_2SO_4, (11) K_2SO_4. From SPENCER (13).

required to accomplish the change, the solution is unsaturated and the aqueous vapor pressure is increased. This results in a temporary increase of the relative humidity. The average change of solubility in 100 gm. of water for each degree change in temperature between 0 and 100 is 0.04 gm. for NaCl and 1.07 gm. for $NaNO_3$. Because of

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the smaller change in solubility, therefore, NaCl would be the salt preferred. Another advantage in using NaCl is that the aqueous vapor pressure in a saturated solution of this salt is affected much less by changes in temperature than is that in a $NaNO_3$ solution. As a result of the variation in aqueous vapor pressure, the relative humidity above a NaCl solution changes from 77 to 75 per cent between 10° and 40° C., which are the usual extreme temperatures of incubation. In the case of $NaNO_3$, the relative humidity obtained is 80 per cent at 10° and changes to 72 per cent at 40° C. The vapor pressure of the salts is so small that this factor is of no importance, and need not be considered in evaluating the method.

Physical control of humidity

Physical control is achieved by electrical or mechanical regulation of the rate of humidification. If vaporization of the water within the humidifier proceeds continuously, the air stream will be saturated, whereas intermittent vaporization results in the air being saturated only part of the time.

Continuous vaporization

The method of continuous vaporization is easily applied if a saturated atmosphere is desired, but it is rather difficult to use when relative humidities of less than 100 per cent are required. In closed chambers the air can readily be saturated by evaporation from a free water surface or from wet strips of cloth (**22**). This procedure, although simple, has the disadvantage that the water from the evaporating surface will condense on the experimental material and on the sides of the container unless the temperature is held absolutely constant. A simple variation which permits the use of fresh-air chambers is to blow the air through a humidifier containing water (**12**). When the velocity of the air stream is small, a glass tower containing wet cloth can be used for humidification (fig. 5). To saturate large volumes of air, a water spray is often employed (**2, 7, 8, 14**).

By this method two procedures are available to secure relative humidities less than saturation. One way is to saturate the air at a low temperature and then reheat it. The relative humidity ob-

tained will depend on the initial and end temperatures (fig. 10). This method is applicable in commercial work but the large amount of complicated control equipment required generally makes it impractical for use in culture experiments. The second way is to mix saturated air in definite proportions with dry air or with air of a fixed relative humidity. The air can be dried by passing it through

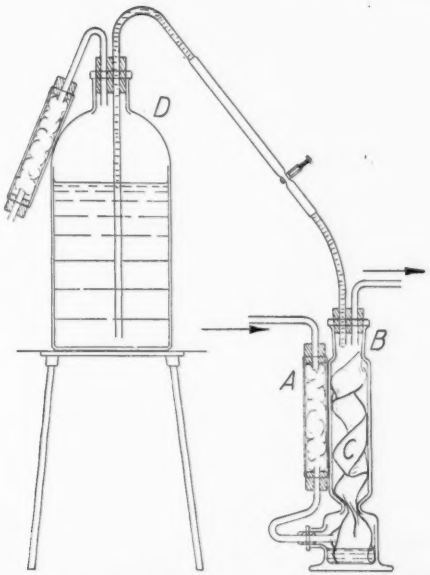

FIG. 5.—Apparatus for saturating air stream under sterile conditions: *a*, cotton filter; *b*, humidifier containing wet cloth (*c*); *d*, water supply. Outlet from humidifier leads to chamber which contains the experimental material.

towers containing a desiccating agent. Anhydrous magnesium perchlorate (anhydrone) and anhydrous barium perchlorate (desicchlora) are both suited to this purpose, because they absorb a relatively large proportion of water without liquefying. Anhydrous calcium chloride, phosphorus pentoxide, and silica gel are also used to desiccate air. Another way of drying air is to pass it over freezing coils. The moisture is precipitated from the atmosphere at a low temperature, and the air, when reheated, is almost dry. Air which

has been saturated at $-10°$ C., for example, will have a relative humidity of about 9 per cent at 25° C. The dry and moist air streams may be combined in definite proportions by means of mixing valves, and if the temperature is controlled, any desired relative humidity can be obtained. Two precautions which must be taken in the application of this procedure are the careful regulation of the temperature and the use of accurate mixing valves.

Intermittent vaporization

The most widely used physical method of humidity regulation is intermittent vaporization (**1, 2, 3, 7, 8, 11, 14, 16, 21**). This method is much more complicated and expensive than are the chemical procedures, but it has the advantage that chemical contamination of the cultured organisms is impossible, since only pure water is used. The employment of this method permits free circulation and renewal of air within humidity chambers of large capacity. When the desired moisture content of the atmosphere has been attained, humidification is interrupted, either electrically or mechanically, by a hygrostat contained within the humidity chamber. This instrument may operate in one of two ways, lineametrically or psychrometrically.

The regulating mechanism of a lineametric hygrostat is a material which increases in length owing to imbibition of atmospheric moisture. Human or animal hairs (**14, 16, 21**) are commonly used for the purpose, since they react quickly to small changes in relative humidity. Wood strips are used as the expanding medium in humidity-control apparatus designed for operation in large volumes, such as rooms. These instruments show a greater lag in reaction than do hair hygrostats. A simple hair hygrostat with electrical control can be constructed rather inexpensively (fig. 6). The hairs of the hygrostat actuate a lever at the end of which is a tungsten or platinum contact that dips into a mercury cup. The hygrostat works on a low-voltage battery circuit which operates the humidifier through a relay. One contact wire leads into the mercury cup and the other wire connects to the standard which supports the lever bearings. In using this apparatus it has been found that the surface of the mercury gradually oxidizes. The oxidized surface is easily skimmed off, however. Hygrostats of this type, unless carefully

designed, require occasional readjustment, because of the strain exerted on the hairs by the weight of the lever.

Construction of the psychrometric hygrostat is based on the cooling effect of evaporation from a moist surface (2). The evaporating surface is a linen cloth which is wrapped around the bulb of a mercury thermometer (fig. 7). The cloth is kept moist by absorption of distilled water through a wick from a reservoir placed below the thermometer bulb. The hygrostat is connected in series with the coil of a relay, the armature of which is in the humidifier circuit.

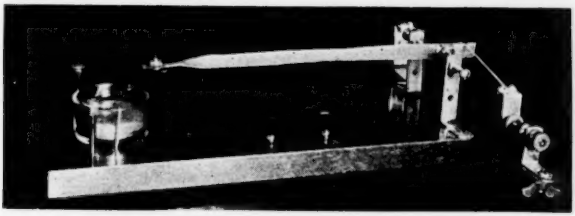

FIG. 6.—Hair hygrostat with electric contacts

One contact wire is sealed into the bulb of the hygrostat, and the other wire, made of platinum, extends down the neck to the surface of the mercury. Oxidation of the mercury surface, due to sparking, is avoided by filling the neck with castor oil or an oil of similar quality. The instrument is regulated to respond at a definite humidity by adjusting the wire in its neck to touch the mercury meniscus at a point corresponding to the desired reduction in temperature. A bimetallic-strip thermostat wrapped in cloth could be substituted for the mercury thermometer.

The two characteristics which determine the accuracy of a psychrometric hygrostat are sensitivity and lag. Sensitivity of the psychrostat depends on the ratio of the bore of the neck to the capacity of the bulb. Since the minimum bore is limited by the diameter of the wire, sensitivity can be increased only by using a bulb of large capacity. However, a large bulb will cause an excessive lag in the instrument. In the design of a hygrostat, therefore, a suitable compromise must be established between these two features.

The advantage of a psychrometric hygrostat is that no readjust-

ment is required after the first setting; it is necessary only to replenish the distilled water in the reservoir. This type of instrument possesses two undesirable features, however, which are not found in the hair hygrostat. Firstly, continuous air movement is necessary for its correct operation, whereas the hair hygrostat operates as efficiently in still as in moving air. The second undesirable feature concerns the necessity of careful temperature regulation. The relative humidity secured by the use of this instrument depends on the difference between the predetermined temperatures of the wet bulb and of the air. If the air temperature varies, the difference between it and that of the wet bulb will be changed and a relative humidity other than the desired one will be attained. A change in relative humidity of approximately 6 per cent is induced by a 1° variation when the air temperature is 25° and the wet bulb is set at 20° C. The effect of variations in air temperature on the accuracy of the humidity control depends on the temperature and relative humidity of the air within the chamber. At low temperatures and at high humidities, variations in temperature have the greatest effect. Temperature control must be accurate, therefore, if close humidity

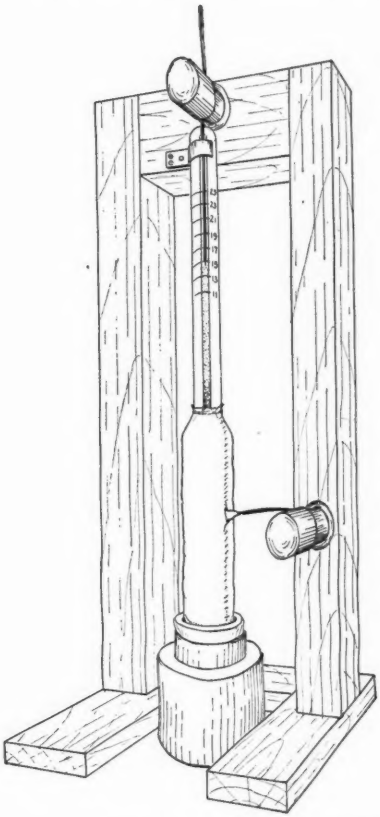

FIG. 7.—Psychrometric hygrostat with electric contacts.

regulation is to be attained with a psychrometric hygrostat. The lineametric hygrostat, on the contrary, operates independently of the air temperature since the hairs respond directly to changes in relative humidity.

In the case of control by intermittent vaporization, the air passes through the humidifier into the humidity chamber. When the humidity chamber is small, and when the volume and velocity of the incoming air are relatively great, the air may be humidified at room temperature by passive vaporization from a water surface. Under these conditions a simple humidifier is sufficient. Moistened cloth in a glass tower is suitable for this purpose. Humidification is stopped by interruption either of the air current or of the water supply (**14, 21**). When the volume of the humidity chamber is great, however, humidification at a temperature considerably above that of the air within the humidity chamber is desirable. Since the moisture content of the air at saturation is considerably increased by heating, the atmosphere of the humidity chamber can be humidified quickly by this means. Increasing the water-holding capacity of the humidifying air stream by heat is referred to in this paper as forced vaporization.

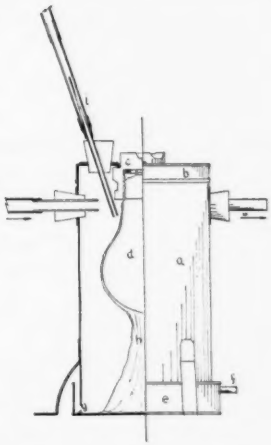

FIG. 8.—Humidifier of forced-vaporization type: *a*, exterior of metal cylinder; *b*, cover; *c*, electric light socket; *d*, electric bulb; *e*, tray; *f*, run-off spout; *g*, outlet from cylinder to tray; *h*, linen wick; *i*, constant-drip tube.

Several kinds of humidifiers operating on the principle of forced vaporization have been designed for use in incubating chambers (**16, 21**). A simple one, which was adopted in principle from that designed by STOUGHTON (**16**), has proved satisfactory. It consists (fig. 8) of a sheet-copper cylinder, 8 inches high and $4\frac{1}{2}$ inches in diameter, which is fitted with a tight cover. An electric light socket and bulb are so secured in the cover that the bulb is suspended

within the humidifier and the leads of the socket are external. The cylinder is set in a shallow copper tray, 5 inches in diameter and $\frac{1}{2}$ inch in height, near the top of which is a run-off spout. One-quarter of an inch above the base of the cylinder is a $\frac{1}{8}$-inch hole which serves as a water outlet from the interior of the cylinder to the tray. A wick made of coarse linen is sewed around the electric light bulb and extends to the bottom of the humidifier. A constant-drip glass tube is set through the rubber stopper in the top cover of the humidifier

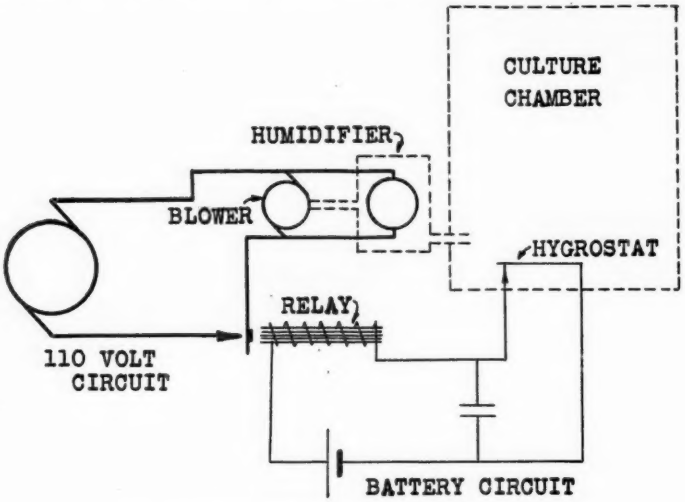

FIG. 9.—Electric hook-up for intermittent vaporization

so that the water drips on the wick near the base of the bulb. About $1\frac{1}{2}$ inches below the top of the humidifier, two $\frac{3}{4}$-inch holes are drilled opposite each other. Air is blown into the humidifier through one of these openings and the other is connected by means of asbestos-wrapped glass tubing to the culture chamber. The air blower and the bulb of the humidifier are connected in series on a 110-volt circuit, which is controlled through the relay by the hygrostat (fig. 9). The size of the humidifier bulb is determined by the amount of vaporization desired; the larger the bulb the more rapid is the rate of humidification.

Although forced intermittent vaporization is the only practical means of humidity regulation in large chambers, the mechanical nature of the control equipment causes a perceptible lag in the instruments. The factors which cause this condition are such that the method gives good results only at ordinary incubating temperatures (fig. 11, *d*). Efficient control of the relative humidity at temperature extremes is difficult to obtain without the use of equipment which is considerably more complex than that just described. The reason for the impossibility of securing effective humidity control at extreme low and high temperatures is evident when some of the characteristics of atmospheric humidity are considered.

The relative humidity depends on three factors: absolute humidity, temperature, and atmospheric pressure. The latter factor is of such slight importance in humidity-control apparatus that it need not be considered further in the present discussion. The absolute humidity of a saturated atmosphere depends on the vapor pressure, which increases rapidly with a rise in temperature. The warmer the air, therefore, the more concentrated is the moisture in the atmosphere over a freely evaporating water surface. At 0° C., saturated air contains about 4.8 gm. of water per cubic meter, at 25° it contains 23 gm., and at 50° almost 85 gm. (fig. 10). Relative humidity is a percentile expression of moisture content based on the absolute humidity of saturated air. In the case of saturated air at 0° C., 1 per cent of the absolute humidity is 0.048 gm. per cubic meter. If it is assumed, for the sake of illustration, that the capacity of the humidity chamber is $\frac{1}{3}$ cubic meter, then a 5 per cent variation in relative humidity represents a change of 0.08 gm. of water vapor. If humidifying apparatus similar to that described is used, this amount of moisture will be discharged into the atmosphere very rapidly. By the time the actuating part of the hygrostat reacts, the relative humidity might be increased by 10 or 20 per cent. With the common methods of control, therefore, it is impossible to humidify the air slowly enough at low temperatures to prevent a considerable lag in the response of the hygrostat. The higher the temperature, the less important is the lag of the hygrostat. This is due to the larger quantity of vapor required to effect a unit change in relative humidity. The hair hygrostat and humidifier which have been described

function acceptably well above 15° C. Below this temperature, attempts to secure accurate humidity control were unsuccessful.

At high temperatures, the difficulty in securing effective humidity control is due not to the hygrostat, but rather to the humidifier. The moisture holding capacity of the atmosphere increases rapidly

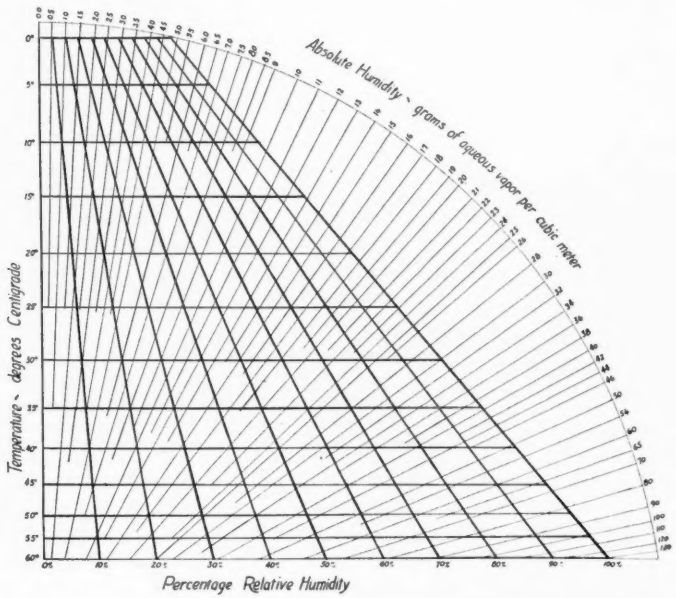
Absolute Humidity - grams of aqueous vapor per cubic meter
Temperature - degrees Centigrade
Percentage Relative Humidity

FIG. 10.—Temperature-absolute humidity-relative humidity diagram. Data calculated from MILLIKAN (10).

at the higher incubating temperatures. A slight variation in temperature will therefore induce a considerable change in moisture holding capacity of the air. If it is assumed, for the sake of illustration, that the relative humidity in a $\frac{1}{3}$ cubic meter chamber is to be held at 80 per cent, it would be necessary in order to counteract the effect of a temperature change from 58° to 62° C. to discharge an additional 6 gm. of water into the air. The rate of humidification with the type of apparatus described is too slow to counteract the effect of this 4° temperature rise. As the temperature falls, the rela-

tive humidity may go as much as 10 per cent above the desired amount (fig. 11, *a*). The incubating temperature which is employed in culture studies of fungi does not generally exceed 40° C., however, and is usually about 30°. At these temperatures, humidity control

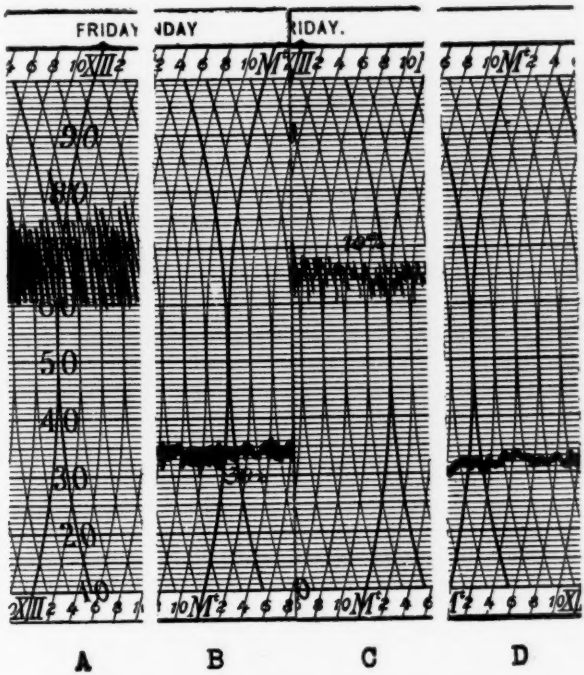

FIG. 11.—Hygrograph records obtained in chambers humidified by intermittent vaporization with hair hygrostat and humidifier of forced-vaporization type: *a*, 67 per cent relative humidity at 52° C. showing excessive variation at high temperatures; *b*, 33 per cent relative humidity at 52° C.; *c*, 67 per cent relative humidity at 38° C. (hygrostat in latter chamber only, humidified air stream being divided equally into the two chambers); *d*, effective control at 25° C. and 33 per cent relative humidity.

with the described apparatus is successful since the rate of vaporization is sufficiently slow to permit the efficient operation of the hygrostat, and is rapid enough to counteract the effect of temperature variation.

When humidity chambers are operated in sets, it has been found possible to simplify the control apparatus considerably. If within two chambers of equal volume the temperature is held at different levels and the humidified air stream is divided, half being blown into each chamber, the relative humidities obtained will be an inverse function of the temperatures. For example, if a hygrostat in one chamber which is operated at 38° C. is regulated so as to secure a relative humidity of 67 per cent, the relative humidity in the other chamber can be calculated from the temperature. Since equal amounts of moisture are discharged into both chambers, the absolute humidity in each will be the same, 30.6 gm. per cubic meter (fig. 10). If the second chamber is operated at 52° C., the relative humidity in it will be 33 per cent. In tests which were run under these conditions, satisfactory humidity regulation was obtained in both chambers, although only the one set of control apparatus was used (fig. 11, *b*, *c*).

Summary

1. Atmospheric humidity can be controlled in culture studies by quantitative and qualitative chemical methods, and by physical methods involving continuous and intermittent vaporization.

2. The quantitative method of chemical control is convenient for small closed chambers. It is easily set up and is inexpensive.

3. The method of qualitative chemical control is used for the regulation of humidity in both closed and fresh air chambers. This method is simple, and requires very little equipment and care.

4. Physical control by means of continuous vaporization is useful to secure a saturated atmosphere. To secure relative humidities intermediate between 0 and 100 per cent, the additional apparatus required generally makes the method impractical for use in culture studies.

5. Intermittent vaporization is the most useful method for large chambers. Control is by means of a hygrostat and a humidifier.

6. Hygrostats are of two types, lineametric and psychrometric. The first acts independently of temperature variation, but requires frequent readjustment unless well designed. The second can be used only when the air temperature is accurately controlled. Under these

conditions, it is more satisfactory than the lineametric hygrostat since readjustment is not required.

7. Humidifiers operate by either passive or forced vaporization. Those which operate on the principle of passive vaporization are used with chambers of small capacity and at low temperatures; those of the forced vaporization type are employed to humidify large quantities of air and for control at high temperatures.

8. Humidity control by physical methods is effective between temperatures of 15° and 40° C. Beyond these extremes, technical difficulties result in decreased accuracy of the humidity control.

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DEPARTMENT OF FOREST BOTANY AND PATHOLOGY
NEW YORK STATE COLLEGE OF FORESTRY
SYRACUSE, NEW YORK

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ANATOMICAL STRUCTURE OF STEMS IN RELATION TO THE PRODUCTION OF FLOWERS

OCRA CHRISTINE WILTON AND R. H. ROBERTS

(WITH FORTY-THREE FIGURES)

Introduction

In connection with the problem of reproduction in higher plants, it is important to determine whether the phenomenon of reproduction has a common physiological basis for a wide range of plants or whether each variety has its own particular physiological condition accompanying the production of blossoms. Although wholly different external environments are required to induce blossoming in different species and varieties as illustrated by long- and short-day plants (**6, 7**), it is possible that a common internal condition may accompany reproduction. Should this be demonstrated, it would greatly aid in the solution of the important practical and technical question of why plants blossom.

LOEW (**10**), FISCHER (**5**), and others have considered this point when proposing a nutritional basis for sexual reproduction. The suggestion of KRAUS and KRAYBILL (**9**) that fruitfulness depends upon the relationship between the carbohydrate and nitrogen composition of the plant also proposes a physiological basis for fruitfulness. FINCH (**4**), NIGHTINGALE (**12**), and ROBERTS (**13**) have presented data which were interpreted as supporting the carbohydrate-nitrogen hypothesis, while ARTHUR and coworkers (**1**) and WORK (**17**) interpret their data as contradicting the existence of such a relationship. There is an ever increasing body of evidence to show a positive correlation between the character of growth of a plant and its reproductive condition (**14**).

Although chemical studies to date have apparently left the matter unsettled, it is possible that other methods of approach can provide more definite data. A study of carbon dioxide exchange in a number of species has indicated that a common physiological condition of the plant accompanies reproduction (**15, 16**). The present report

gives the results of observation made in a number of species to determine the relation between certain anatomical characteristics in the stem and the production or non-production of flowers.

Although anatomical studies have been made upon plants grown under different environmental conditions, there has been little emphasis upon the possible correlation between anatomy and reproduction. DEATS (3) reports that tomato and pepper plants which produced few or no flowers in a short-day environment have more pith and less xylem and bast than plants grown in a long-day environment. The walls of the cells of the xylem and bast are thinner, and although there are fewer large vessels, their average diameter is larger in the non-flowering plants than in comparable cells and tissues of the plants which produced flowers. FINCH (4) notes that the xylem of the fruitful growth of biennially bearing apple trees shows the most summer wood, including a relatively large number of parenchymatous cells. The unfruitful terminal growth has the least amount of summer xylem and the proportion of fibers to parenchyma is high.

Materials

In attempting to determine a possible correlation between the anatomy of stems and the production or non-production of flowers, a wide range of species was examined during a period of five years. A comparison was made of the flowering and non-flowering stems of twenty-eight species of plants belonging to fourteen families, as follows:

Amaranthaceae: *Amaranthus retroflexus* L.

Aizoaceae: *Tetragonia expansa* Murr.

Caprifoliaceae: *Sambucus canadensis* L.

Cruciferae: *Cheiranthus cheiri* L., *Mathiola incana* R. Br.

Geraniaceae: *Pelargonium domesticum* Bailey, *P. peltatum* Ait.

Linaceae: *Linum usitatissimum* L.

Euphorbiaceae: *Euphorbia pulcherrima* Willd., *Ricinus communis* L.

Begoniaceae: *Begonia semperflorens* Link and Otto.

Onagraceae: *Clarkia elegans* Dougl., *Fuchsia hybrida* Voss.

Apocynaceae: *Vinca major* L.

Labiateae: *Coleus blumei* Benth., *Salvia splendens* Ker.

Solanaceae: *Datura stramonium* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Petunia hybrida* Vilm., *Schizanthus wisetonensis* Low., *Solanum tuberosum* L.

Scrophulariaceae: *Antirrhinum majus* L.

Compositae: *Ageratum conyzoides* L., *Calendula officinalis* L., *Chrysanthemum morifolium* Ram. var. Lillian Doty, *Cosmos sulphureus* Cav. var. Klondike, *Zinnia elegans* Jacq.

Except in the case of flax, the plants from which the material was obtained were grown under controlled conditions in the greenhouse, photoperiod being the variable factor used to modify or regulate the production of flowers.

The flax was grown in the garden during the summer of 1931. Prior to flower production, samples of stems were taken which were classed as non-flowering. Later, when flowers were present, samples of "flowering" stems were taken. At this time, one row of plants was shaded with 8-oz. burlap and given a mixture of ammonium sulphate and sodium nitrate (two-thirds $(NH_4)_2SO_4$ and one-third $NaNO_3$), applied broadcast as nearly uniformly as possible at the rate of 500 pounds per acre. These plants became rather weakly vegetative and produced new branches. After a time the untreated plants also began a "second growth," as indicated by the development of many new branches. Samples of the stems were taken from both groups of plants below the point of origin of the new growth.

The remaining species are represented by samples of stems of flowering and non-flowering plants which were growing in the University greenhouses and gardens. This type of material was used for the preliminary study, and in all instances the observations indicated that the anatomical characteristics were correlated with the reproductive stage rather than with the cultural conditions or age of plant. The present report, however, is limited to material from the controlled plants.

In addition to samples from strictly flowering and non-flowering plants, samples were taken of the stems of plants of cosmos, chrysanthemum, and zinnia on which flower buds were first appearing. "Budding" plants were considered a transition stage from a non-reproductive to a distinctly reproductive type of growth.

Plants which had changed from a reproductive to a non-produc-

tive type of growth, either by inducing abortion of blossoms or by the production of vegetative shoots following the development of flowers, were also examined. The abortion of flower buds was induced in plants of cosmos by the transfer of budding plants from a short- to a long-day environment, while the opposite change from a long- to a short-day environment was necessary to induce the abortion of flower buds on plants of petunia. Flowering plants of poinsettia which were transferred to a long-day environment developed vegetative shoots. Samples were taken from the internode below the topmost vegetative shoot.

Two species, cosmos and *Ricinus*, were induced to produce a second set of flowers following a period of vegetative growth. Samples of this material were taken as an additional means of checking how closely the anatomy might be associated with the external character of growth throughout a flowering phase, a non-flowering phase, and a second flowering phase.

It is commonly stated that the usual woodiness of plants at the time of flowering is a result of the seasonal maturity which generally accompanies the production of flowers or is in fact the result of flowering itself. In order to determine whether or not this condition of "maturity" is a result of blossoming or one which precedes fruiting and is inherent in the reproductive stage, plants of cosmos and petunia were disbudded in order to prevent the development of flowers. It seemed that if deflorated plants developed a "mature" structure in contrast to the situation in vegetative non-blossoming plants, this would be evidence of a positive relation between stem structure and reproductive condition. The disbudding procedure consisted of the removal of flower buds about twice a week, while they were very small but after they were sufficiently differentiated to be distinguished from any foliar primordia which might be present.

Methods

Samples of flowering stems were taken from the second internode below terminal inflorescences as in cosmos. Where there was a vegetative growing point above the flower, as in petunia, samples were taken from the fourth internode below the youngest flower. Samples of non-flowering stems were taken from the fourth elongated inter-

node from the tip of the stem, a region in which secondary tissues are being differentiated.

Preliminary observations were made from free-hand sections of the stems. The sections were stained with an aqueous solution of iodine-potassium iodide to which had been added an equal volume of 1 per cent eosin in 95 per cent alcohol. This combination gives a better distinction between wall and cell content in material which contains little or no starch than is obtainable by the use of iodine-potassium iodide alone. The sections were then mounted on slides in glycerin containing iodine-potassium iodide.

For permanent mounts, the samples of stems were fixed in formalin-acetic-alcohol (6 cc. formalin, 2 cc. glacial acetic acid, and 100 cc. of 50 per cent alcohol). Both ethyl alcohol and butyl alcohol were employed at different times as dehydrating agents. The fixed material was imbedded in paraffin and cross sections 15–20 μ in thickness were made. In the earlier work, safranin and light green were used for staining the sections but later a combination of aqueous crystal violet and erythrosin in clove oil was found to be very satisfactory for differentiation of the tissues. These last two stains were less satisfactory for photomicrographs, however, and when photographs were desired the sections were stained with cotton red alone.

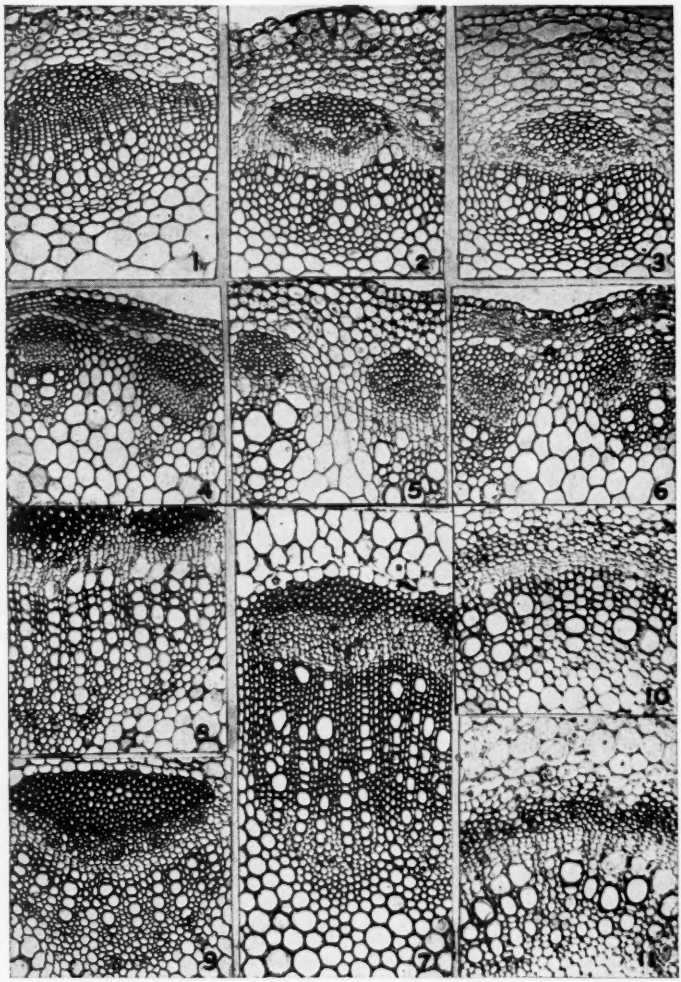
Observations and discussion

A number of questions arose in the course of these studies. The first one to be considered may be stated thus: Are there consistent anatomical differences in the stems of plants producing flowers and of plants not producing flowers?

It was found that there were characteristically different conditions in the flowering and non-flowering stems of the various plants examined. These differences were more pronounced in some species than in others. Chrysanthemum will be used as an illustration of a plant having a markedly distinctive anatomical structure in the flowering stem (figs. 1, 14).

There appear to be no meristematic cells present in the cambial region.[1] All the cells of the pericycle and phloem, except a few of the

[1] The leaf traces commonly have a relatively inactive cambium in both flowering and non-flowering stems. These have not been included in the characterization of the anatomy of the various samples.



FIGS. 1–11.—Photomicrographs of portions of transverse sections of stems: Figs. 1–3, chrysanthemum (1, from a flowering plant; 2, from a non-flowering plant; 3, from a budding plant). Figs. 4–6, cosmos (4, from a flowering plant; 5, from a non-flowering plant; 6, from a budding plant). Figs. 7–9, zinnia (7, from a flowering plant; 8, from a non-flowering plant; 9, from a budding plant). Figs. 10–11, salvia (10, from a flowering plant; 11, from a non-flowering plant). ×100.

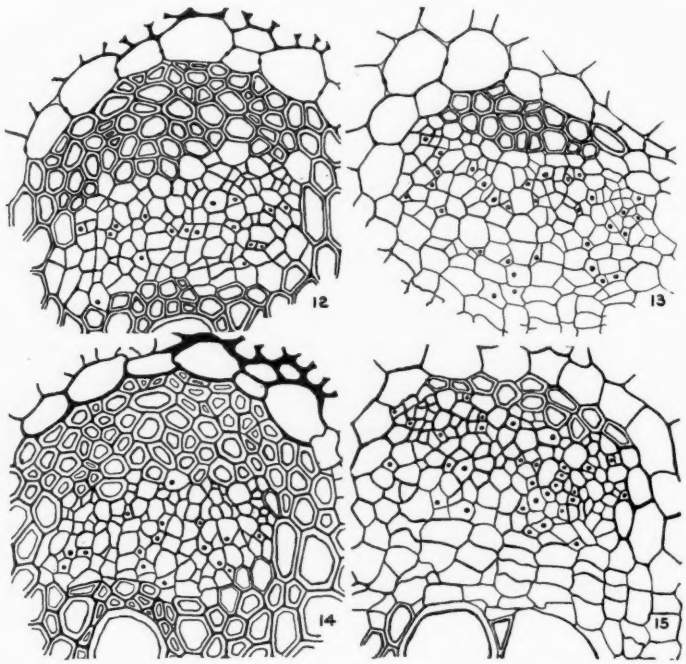
sieve tubes and companion cells, have become thick-walled and are stained by the basic dye, crystal violet. The last formed secondary xylem consists entirely of small, thick-walled, usually oblong elements which appear to be of one type when seen in transverse section. When these elements are examined in longitudinal sections they are found to be of at least two types, some having tapering end walls while others have almost square end walls. All are more or less conspicuously pitted. The xylem and entire perimedullary zone are also stained by crystal violet.

In contrast to these characteristics, the comparable internode of a non-flowering stem of chrysanthemum (figs. 2, 15) shows an active cambial zone four to six cells in width, which is composed of typical thin-walled elements. Numerous xylem vessels as well as companion cells and sieve tubes are in process of differentiation. Fewer of the cells of the pericycle have thickened walls than is the case in the flowering stem, although the pericyclic fibers have become fully as thick-walled as corresponding ones in the flowering stem. In the non-flowering stem, only the walls of the pericyclic fibers and the fibers of the phloem and the xylem vessels are stained by crystal violet, the remaining cells being stained by erythrosin. The smaller amount of tissue in the non-flowering stem in contrast to that of the flowering stem which is stained by the basic dye is to be noted.

Sections from stems of plants of chrysanthemum which were just beginning to develop flower buds show a structure intermediate between that of the flowering and non-flowering stems (fig. 3). There is a cambial zone present but it averages only two cells in width. As is characteristic of the flowering stem, small xylem elements, which are oblong in section view, are being differentiated on the inner side of the cambium.

The anatomical characteristics of the flowering, budding, and non-flowering stems of cosmos closely parallel those observed in the comparable stems of chrysanthemum. Both species belong to the same photoperiodic group and require less than fourteen hours of light per day for the production of flowers. The only indication of cambium in the flowering stem of cosmos (figs. 4, 12) is the presence of a few meristematic cells, generally three or four per bundle. The secondary xylem adjacent to the cambium is made up of small,

thick-walled cells. The walls of the cells of the pericycle as well as of those of the perimedullary zone have become thickened. Many of the older cells of the phloem have also become relatively thick-walled. The non-flowering stem of cosmos (figs. 5, 13) has a relatively wide cambial zone, and in the xylem numerous vessels of large



FIGS. 12–15.—Camera lucida drawings of portions of bundles from flowering and non-flowering stems: Figs. 12, 13, cosmos showing relatively larger elements in youngest phloem of non-flowering stem (fig. 13) when compared with those of flowering stem (fig. 12). Apparent absence of meristematic cells in cambial zone seen in fig. 12. Figs. 14, 15, chrysanthemum showing characteristics similar to those noted for cosmos in figs. 12 and 13.

lumen and many thin-walled parenchymatous cells are in process of differentiation. Both the interfascicular pericycle and the perimedullary zone are composed of relatively thin-walled cells. The vascular bundles in the stems of the budding plants of cosmos (fig. 6) show

a narrow fascicular cambial zone, and sometimes there is still a suggestion of interfascicular activity. The cells of the pericycle and perimedullary zone have, in general, thinner walls than comparable cells in the flowering stems. The small secondary xylem elements which are characteristic of flowering stems are again present.

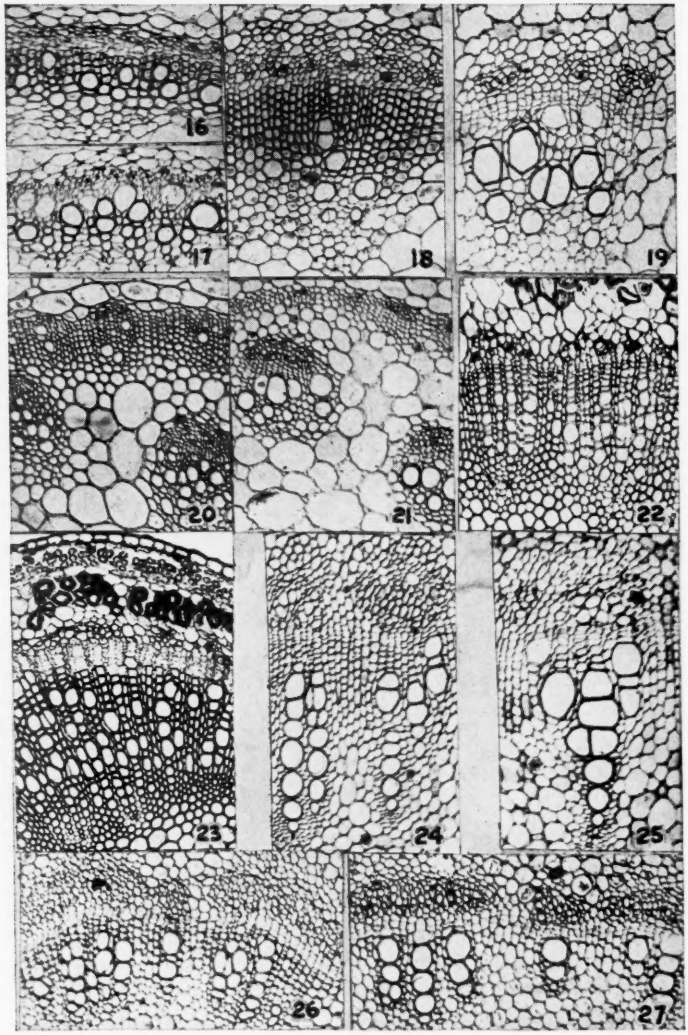
Sections of the phloem of flowering and non-flowering stems of cosmos (figs. 12, 13) show that the cells of the secondary phloem of the non-flowering stem are relatively large in transverse section compared with those of the primary phloem, and the cells of the secondary phloem in the flowering stem appear to be little larger than the cells of the primary phloem. The whole region in the flowering stem has cells with thicker walls. Sections from stems of chrysanthemum show similar anatomical characteristics (figs. 14, 15).

In salvia, another short-day species, the structure of the flowering stems is somewhat less distinctive than that of chrysanthemum and cosmos. The flowering stems show a relatively wide cambial zone, but the last formed secondary xylem consists of small thick-walled cells with only a few large vessels in process of differentiation (fig. 10). The non-flowering stem of this species has a distinctly different appearance, however, with a wider, more active cambial zone than the flowering stem; and the secondary xylem is composed of thin-walled parenchymatous cells and numerous large vessels (fig. 11).

Sections from flowering and non-flowering stems of *Amaranthus retroflexus* (figs. 20, 21) and poinsettia (figs. 26, 27) also have anatomical characteristics which have been described as typical of the respective types.

A short-day environment was needed to induce blossoming of the plants already discussed. Examination of a long-day and an indeterminate (6) type shows that the anatomical condition is not related to the photoperiod needed to initiate blossoming, but to the reproductive condition of the plants. This fact is illustrated by the sections of *Zinnia elegans* (figs. 7–9), New Zealand spinach (figs. 16, 17), *Coleus blumei* (figs. 18, 19), and flax (figs. 22, 23).

The flowering and non-flowering stems of *Ricinus* (figs. 24, 25) are of particular interest because the phloem elements as well as the xylem elements seem to show a marked correlation with the character of growth. The phloem elements which are in process of differ-

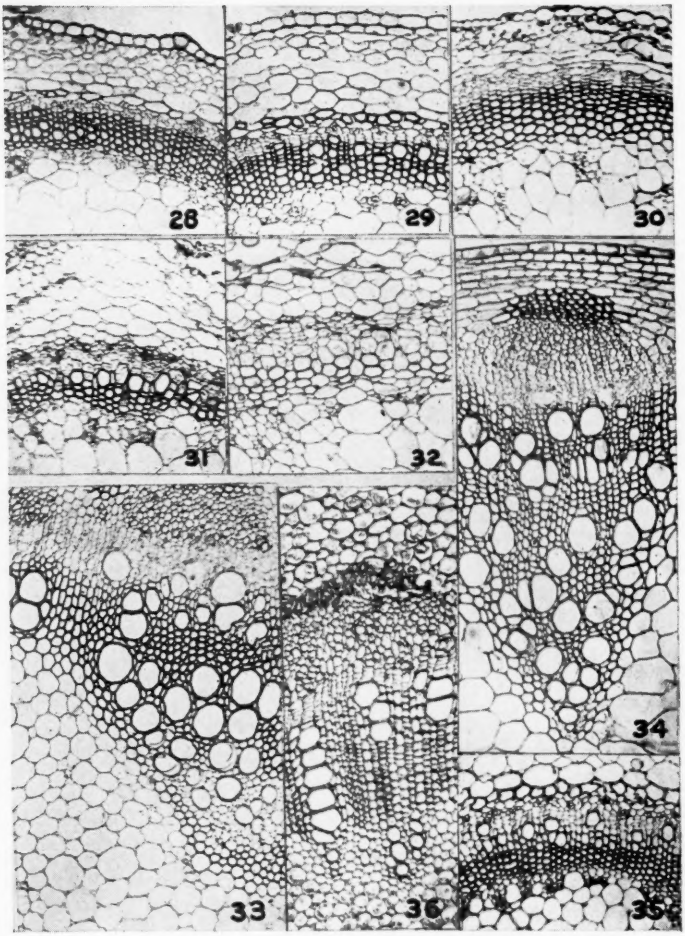


FIGS. 16–27.—Photomicrographs of portions of transverse sections of stems: Figs. 16, 17, New Zealand spinach (16, from a flowering stem; 17, from a non-flowering stem). Figs. 18, 19, coleus (18, from a flowering stem; 19, from a non-flowering stem). Figs. 20, 21, *Amaranthus retroflexus* (20, from a flowering stem; 21, from a non-flowering stem). Figs. 22, 23, flax (22, from a flowering stem; 23, from a non-flowering stem). Figs. 24, 25, *Ricinus* (24, from a flowering stem; 25, from a non-flowering stem). Figs. 26, 27, poinsettia (26, from a flowering stem; 27, from a non-flowering stem). ×100.

entiation in the non-flowering stem are relatively large in cross-sectional diameter, while in the flowering stem a few large elements have differentiated but the ones adjacent to the cambial zone are small in diameter.

Sections from the stems of plants of petunia in which flowering was only slightly delayed by a short-day environment as compared with plants which were grown with sixteen hours of light per day have an anatomical structure which suggests the flowering condition, even though flowers had not yet been produced (figs. 28, 29). There is a cambium present in both instances although it appears to be slightly less active in the flowering than in the non-flowering stem. In figure 32, however, is shown a section from an extremely vegetative, non-flowering plant of petunia which had been grown during the short days of November and December. In this case the structure does not resemble that of a flowering stem, but has the anatomical characteristics of a non-flowering stem. The differences between the flowering and non-flowering stems of plants of *Schizanthus* are greater than those found in petunia (figs. 30, 31).

Before summarizing the results of the observations reported thus far, attention is called to an interesting condition which has a bearing upon the interpretation of the data. This is the extent to which the primordia are utilized in flowering and might be called the degree to which a plant becomes reproductive. Those species, as cosmos and amaranthus, which differentiate flower buds at most of the growing points, usually die following fruiting. In contrast to these, species such as petunia continue to grow vegetatively while flowers are being produced. Flax attains a degree of reproductiveness intermediate between that of cosmos and petunia, in that numerous flowers are produced for a time during which there is little or no vegetative growth, but later there may be a renewal of vegetative activity. In this study it has been observed that cambial activity seems to be distinctly correlated with degree of flowering. With plants on which most of the primordia are reproductive, there is almost a complete absence of cambium. On the other hand, plants which make some vegetative growth while flowering, or which again become vegetative readily, have always shown more or less cambium although not necessarily in an active condition. This question of the relation of



FIGS. 28–36.—Photomicrographs of portions of transverse sections of stems: Figs. 28, 29, petunia (28, from a flowering stem; 29, from a non-flowering stem). Figs. 30, 31, *Schizanthus* (30, from a flowering stem; 31, from a non-flowering stem). Fig. 32, petunia, from a very vegetative, non-flowering plant. Figs. 33–36, sections taken below aborted floral buds (33, portion of section of cosmos taken from internode below aborted bud shown in fig. 37 showing zone of small thick-walled xylem elements followed by zone of relatively thin-walled elements and numerous vessels and active cambium). Fig. 34, section of cosmos taken below aborted floral buds of an old plant. Fig. 35, petunia. Fig. 36, poinsettia. ×100.

cambial activity to renewal of vegetative growth following flowering as well as a consideration of the cambial condition at different levels along stems with many internodes is being made the subject of a separate paper.

Regarding a possible relation of anatomy to reproduction, the evidence is interpreted as showing a positive correlation, since flowering and non-flowering stems of a variety have certain distinguishing characteristics regardless of the age of the plant or the environmental conditions necessary to induce blossoming. The anatomical characteristics observed in the flowering and non-flowering stems may be summarized as follows: (1) The cambium of the flowering stem shows little if any activity while the non-flowering stems have a relatively wide, active cambial zone. (2) The secondary xylem lying adjacent to the cambium in the flowering stems of all the species studied consists mainly of small thick-walled elements and relatively few vessels as seen in section view, while in the non-flowering stem it consists characteristically of thin-walled parenchyma and more numerous vessels. (3) A greater number of the cells of the pericycle and of the perimedullary zone have thicker walls in the flowering than in the non-flowering stem. (4) In the flowering stem, the pericycle, perimedullary zone, and certain elements of the xylem tend to stain with basic dyes to a greater extent than do the comparable regions and elements in the non-flowering stem. (5) The phloem region of the flowering stems has relatively thick-walled cells as compared with that of the non-flowering stems.

The second question to be considered is: Will the elements produced by cambial activity at the time a vegetative non-flowering period of growth occurs following the production of flowers be characteristic of a flowering stem or will they resemble those of a non-flowering stem?

Figure 37, right, illustrates the type of floral abortion which occurs when budding plants of cosmos are transferred from a short- to a long-day environment. The time required to effect the change varies with the degree of differentiation of the flower buds at the time the photoperiod is altered. Plants which have well developed buds or open flowers when placed in a long-day environment may continue to produce blossoms for varying lengths of time and finally die in-

stead of renewing vegetative growth. The anatomical structure in the internode below the peduncle of the aborted bud is shown in figure 33. There is a wide cambial zone similar to the vegetative stem of figure 5 and numerous vessels and thin-walled xylem parenchyma have been recently differentiated. Previous to this there has been a period in which the xylem elements were practically all small thick-walled cells such as are found in the stems of flowering plants (fig. 4). Figure 34 illustrates the situation in one of a set of

FIG. 37.—Terminal internodes of three plants of cosmos. Left to right: non-flowering plant grown in long-day environment; flowering plant grown in short-day environment; plant bearing aborted floral bud, grown first in short- and then in long-day environment.

plants in which the flowers had been permitted to develop to a more advanced stage before they were transferred to a long-day environment, and consequently required a greater time before there was a return to a vegetative type of growth. In this case there is also an active cambium. However, the change in type of xylem tissue from that characteristic of flowering to that of non-flowering stems has been by a less abrupt transition than in figure 33.

The anatomy found in stems of plants of petunia (fig. 35) on which abortion of floral buds had been induced is similar to that described for cosmos. There is an active cambium, while the last formed xylem

consists of numerous large vessels and thin-walled elements. This type of tissue has succeeded a zone in which the elements resemble those of the flowering stem (fig. 28).

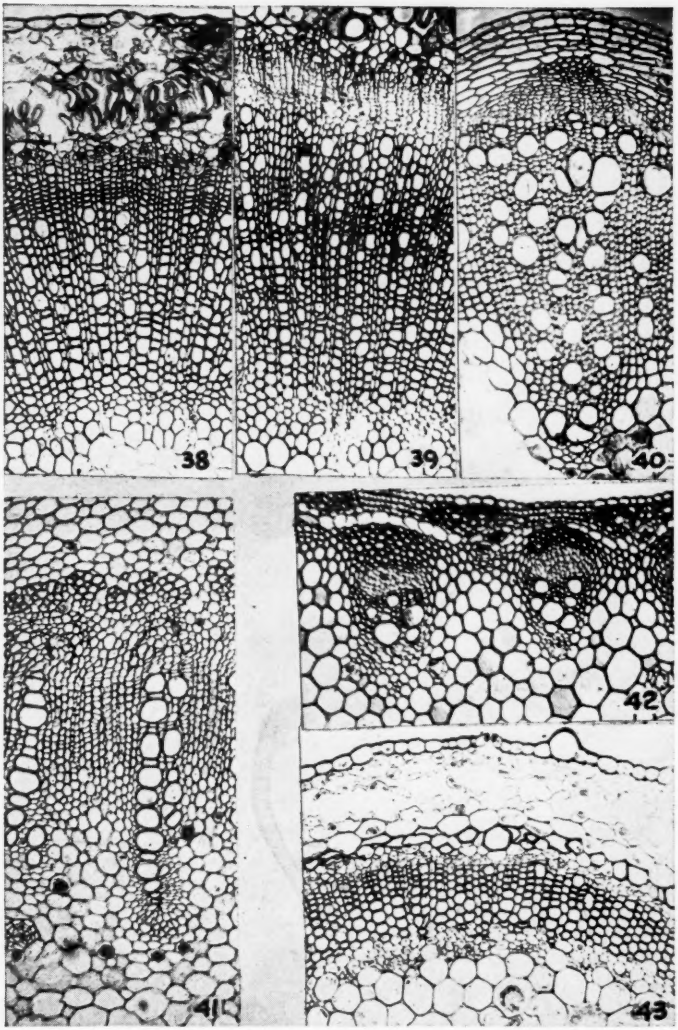
It should be especially noted that the condition of the cambium and the character of the xylem which are associated with the abortion of flowers and the return to a vegetative type of growth are the same for both cosmos and petunia, although a long-day environment was required for cosmos while a short-day environment was necessary for petunia.

Another case in which the anatomical character shifted with the change from a reproductive to a vegetative type of growth can be seen in poinsettia (fig. 36). Since this is a short-day plant, the return to a non-flowering character of growth was induced by a long-day environment similar to that required for cosmos but opposite to that used for petunia.

In the case of flax, the plants became vegetative after mature seeds had been produced. Figure 38 shows a section taken from the stem below the second growth of a plant which had been shaded and was weakly vegetative. In this material the character of the last formed xylem is similar to that which is associated with vegetative type of growth, but the cambium appears to have been only slightly active. Sections from the unshaded plants which were growing vigorously after producing seeds (fig. 39) show a wide cambial zone and there is evidence of considerable cambial activity. The xylem elements being differentiated are also similar to those which are characteristic of non-flowering plants (fig. 23).

Plants of cosmos and *Ricinus* which were producing a second series of blossoms following an intervening period of vegetative growth have stems which show anatomical changes that correspond to the changes in reproductive condition (figs. 40, 41); that is, there is a zone in the xylem in which the elements are larger in diameter and have thinner walls, between an earlier and a present region in which the cells are small and thick-walled as is typical of plants in a flowering condition. The *Ricinus* stem has only a few newly formed cells of the "reproductive" type. It also shows a marked alteration in the character of phloem cells, corresponding to the changes in character of growth from reproductive to vegetative and again to reproductive.

Similar differences in structure have been reported by HAWKINS



FIGS. 38–43.—Fig. 38, section from stem of flax plant which had become weakly vegetative after being shaded with burlap and given a treatment of $NaNO_3$ and $(NH_4)_2SO_4$. Fig. 39, section from stem of flax plant which was making a second period of vegetative growth. Fig. 40, portion of section of cosmos plant which had produced a second set of flowering branches after period of vegetativeness. Fig. 41, *Ricinus*, same as fig. 40 except that second flowering phase was just beginning when sample was collected. Fig. 42, section from stem of disbudded cosmos plant. Fig. 43, same of petunia. ×100.

et al. (**8**) in work on the physiological factors affecting the fruiting of cotton. They noted that rapid vegetative growth, as expressed through the formation of large thin-walled cells in the secondary xylem tissue of the stem, is accompanied in a general way by an increased shedding of bolls. Photographs of sections of such stems of cotton which accompany the report of HAWKINS indicate a change in the structure of the xylem from one resembling "summer wood" to one resembling "spring wood."

Thus in answer to the question of whether or not the elements produced by cambial activity when a vegetative non-flowering type of growth follows the production of flowers will resemble those of a flowering or of a non-flowering stem, the observations upon the five species examined, cosmos, poinsettia, petunia, flax, and *Ricinus*, as well as the report of HAWKINS, indicate that there is a correlation between the anatomy of the stems and the reproductive character of growth of the plants.

The third question for consideration in this study may be stated: Is the type of structure which is characteristic of flowering stems the result of the production of flowers or of a physiological condition which initiates both the production of flowers and the anatomy?

Evidence upon this question was secured by disbudding plants in the early stages of flower formation. Sections of the stems of disbudded plants of cosmos have a structure which resembles that of a flowering stem (fig. 42). They have an apparently inactive cambium and the last xylem elements are small, thick-walled, and tangentially flattened. However, the cells of the perimedullary zone, pericycle, and xylem have even thicker walls than are generally seen in a flowering stem. Sections from disbudded stems of plants of petunia show thick-walled cells in these same regions, although the thickness of the walls is, in general, somewhat less noticeable than in cosmos (fig. 43). The observations made on cosmos and petunia agree in general with those reported by MIRSKAJA (**11**), who removed the flower buds of *Mirabilis jalapa*, *Zinnia elegans*, and *Ageratum mexicanum*. Zinnia and ageratum showed a striking production of lignified material, a phellogen, and an endodermis with Casparian strips. In the present study, sections from stems of disbudded plants of cosmos show that all of the tissues except the central pith cells, cor-

tex, and the youngest phloem elements have markedly thick-walled cells which stain with the basic dyes. The development of an endodermis with Casparian strips seems to be a characteristic of the species rather than being associated with disbudding in cosmos. Casparian strips are frequently observed in the flowering stems and even in the non-flowering stems of this species, and also in chrysanthemum. They have not been observed in stems of either disbudded or flowering plants of petunia.

AUSTIN (2) concluded from his study of exflorated plants that "old age and death in the soy-bean are due to circumstances which accompany the reproductive phase but are not the direct result of it."

The preceding evidence from disbudded plants of cosmos and petunia seems to indicate that the differentiation of the structure of the flowering stem precedes or accompanies and is not a result of flowering. Thus the presence of a zone of thick-walled cells and the decrease of cambial activity of the flowering stem are not due to the production of flowers but to a physiological condition which is responsible for both.

Summary

1. The flowering stems of all the species examined seem to have certain anatomical characteristics in common, regardless of age or of photoperiodic classification.

2. In contrast to the non-flowering stem, the flowering stem is characterized by: (1) a less active cambium; (2) a zone consisting mainly of thick-walled secondary xylem elements lying adjacent to the cambium in contrast to rather numerous vessels and thin-walled parenchymatous cells in the last formed xylem of the non-flowering stems; (3) generally thicker walls of the cells of the pericycle, perimedullary zone, xylem, and phloem; (4) freer staining with "basic" dyes of the pericycle, perimedullary zone, and certain elements of the xylem.

3. Certain specific differences in the flowering stems examined seem to be directly correlated with the "degree of reproductiveness" which the species attains.

4. Xylem elements which are characteristic of the flowering stem

are produced by plants of cosmos and *Ricinus* which were induced to produce a second set of flowering branches following a vegetative period.

5. The consistent differences in structure, which accompany the changes in character of growth as seen in the sections from stems of plants bearing aborting floral buds and of plants producing a second set of flowers following an interval of non-reproductive growth, are offered as evidence that the anatomy of the stem is related to the reproductive character of the plant.

6. The stems of disbudded plants have a structure resembling that of a flowering stem, which seems to indicate that the characteristic structure accompanies and is not a result of the production of flowers.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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ZINC RELATION IN MOTTLE-LEAF OF CITRUS

A. R. C. HAAS

(WITH SEVEN FIGURES)

Introduction

As a result of the work of CHANDLER, HOAGLAND, and HIBBARD (1) on little-leaf or rosette of plum trees, JOHNSTON (6) demonstrated that the application of zinc sulphate to the citrus tree or soil is usually followed by a marked improvement in the condition of the tree. Subsequently THOMASON (9), PARKER (8), and others extended greatly the treatments used by JOHNSTON.

This paper reports data obtained regarding several phases of the problem of the zinc relation in mottle-leaf of citrus: (1) effect of zinc treatment on the disappearance of mottling and on the rooting of mottled cuttings; (2) sugar content of leafy-twig cuttings of Valencia orange in relation to mottling; (3) artificial production of mottle-leaf in soil and sand cultures; and (4) relation of zinc to the presence of mottle-leaf in the early stages of growth in solution cultures.

Investigation

EFFECT OF ZINC SULPHATE ON DISAPPEARANCE OF MOTTLING AND ON ROOTING OF MOTTLED CUTTINGS

These effects were studied by means of cuttings from healthy and from mottled Valencia orange trees grown at the University of California, Citrus Experiment Station. Healthy and mottled cuttings were used as untreated controls, while other healthy and mottled cuttings were dipped in the following mixture: 100 gm. of $ZnSO_4 \cdot 7H_2O$, 50 gm. of $Ca(OH)_2$, a minute amount of blood albumin spreader, and 1 liter of distilled water.

A similar experiment was also set up in which leaf cuttings of Valencia orange were used. These consisted of the whole blade and the petiole without axillary buds or portions of the twigs; hence no new top growth was possible, all growth necessarily having to take place in the root system.

The cuttings were planted in river gravel over a deep layer of crushed rock in bottom-heated (80° F.) rooting chambers. The cuttings were sprinkled with distilled water from a tinned sprinkling can in order to keep the additions of zinc to the untreated control cuttings at a minimum.

The two types of cuttings of Valencia orange were grown in the gravel beds from November 1, 1934 until February 16, 1935, during which time the new shoots of the leafy-twig cuttings were not permitted to develop. On February 16, 1935, the two types of cuttings were removed and the effect of zinc on their rooting was noted (table I). The greater fresh weight of the root systems produced by the zinc-treated mottled Valencia leaves may be due to the greater average fresh weight of these treated mottled leaves.

Although the average fresh weights of the mottled orange leaves are less than those of the healthy leaves, nevertheless the average fresh weights of the root systems of the mottled leaves are greater than those of corresponding healthy leaves.

The healthy Valencia leafy-twig cuttings were grown $3\frac{1}{2}$ months in the rooting chamber. The adhering zinc-mixture residue was wiped from the leaves and the leaves of the control plants also were wiped. The zinc-treated healthy leaves had a deep blue-green color while the untreated healthy leaves had a yellowish or dull brownish green color. In the healthy Valencia leafy-twig cuttings used in these experiments there was a marked improvement in leaf color as a result of the zinc treatment.

The dense stand of zinc-treated mottled Valencia leafy-twig cuttings is shown in figure 1*A*, while figure 1*B* shows the sparsely leafed, untreated (control) mottled cuttings. In the period of the experiment not all of the mottling disappeared from the zinc-treated cuttings. The general color and the number of surviving leaves of the zinc-treated cuttings surpassed those of the untreated control cuttings.

The healthy Valencia leafy-twig cuttings (table I) showed the largest dry weights in the leaves and the smallest in the roots, while the mottled leafy-twig cuttings of orange showed the largest dry weights in the twigs and the smallest in the roots.

An advantage of the zinc treatment of mottled leafy-twig cuttings

FIG. 1.—Mottled leafy-twig cuttings of Valencia orange grown in rooting chamber: *A*, cuttings first dipped in zinc mixture (residues removed by wiping the leaves prior to taking photograph); *B*, cuttings without zinc treatment (leaves also wiped). Note dull color and thin stand of control cuttings.

TABLE I

EFFECT OF ZINC ON ROOTING OF HEALTHY AND MOTTLED VALENCIA ORANGE AND LISBON LEMON LEAVES AND LEAFY-TWIG CUTTINGS

CUTTINGS	TREATMENT DIPPED IN ZINC MIXTURE OR NOT DIPPED (CONTROL)	TOTAL NO. OF CUTTINGS	PERCENTAGE			AVERAGE FRESH WEIGHT PER LEAF (GM.)	AVERAGE FRESH WEIGHT PER ROOT SYSTEM (GM.)	AVERAGE FRESH WEIGHT PER TWIG (GM.)	AVERAGE DRY WEIGHT PER LEAF (GM.)	AVERAGE DRY WEIGHT PER ROOT SYSTEM (GM.)	AVERAGE DRY WEIGHT PER TWIG (GM.)
			ROOTED	LEAVES (DEAD)	ALIVE BUT NOT ROOTED						
Valencia orange											
Healthy leaves	Dipped	50	28	36	36	0.855	0.147				
	Not dipped	50	20	46	34	0.786	0.143				
Mottled leaves	Dipped	50	30	40	30	0.752	0.236				
	Not dipped	50	6	56	38	0.587	0.167				
Healthy leafy-twig	Dipped	50 195*	98	0	2	0.877	0.949	0.663	0.308	0.135	0.271
	Not dipped	50 198*	100	4		0.874	0.920	0.600	0.298	0.137	0.244
Mottled leafy-twig	Dipped	50 228*	100	1		0.629	1.140	0.594	0.195	0.170	0.230
	Not dipped	50 221*	82	20	18	0.617	0.976	0.578	0.212	0.166	0.245

* Leaves on leafy-twig cuttings.

TABLE I—*Continued*

Cuttings	Treatment Dipped in zinc mixture or not dipped (control)	Total no. of cuttings	Percentage			Average fresh weight per leaf (gm.)	Average fresh weight per root system (gm.)	Average fresh weight per twig (gm.)	Average dry weight per leaf (gm.)	Average dry weight per root system (gm.)	Average dry weight per twig (gm.)
			Rooted	Leaves (dead)	Alive but not rooted						
Lisbon lemon											
Healthy leaves	Dipped	54	52	48		1.43	0.464				
	Not dipped	54	26	67	7						
Mottled leaves	Dipped	70	60	39	1	0.787	0.300				
	Not dipped	50	64	36							
Healthy leafy-twig	Dipped	51 206*	90	26	10	1.27	1.24	1.37			
	Not dipped	53 218*	92	22	8	1.19	1.43	1.35			
Mottled leafy-twig	Dipped	60 344*	100	6		0.66	0.988	1.12			
	Not dipped	51 282*	100	3		0.63	1.46	1.26			

of orange is seen in the greater number that rooted and in the greater vigor of the leaves, as evidenced by the few leaves that died.

In table I, if the number of leaves on the Valencia twig cuttings that lived is multiplied by the average fresh weight per leaf, the following total fresh weights of the leaves are obtained: healthy dipped, 171.02 gm.; healthy control, 166.06 gm.; mottled dipped, 141.53 gm.; mottled control, 108.59 gm. These results suggest that the leaves of the mottled Valencia leafy-twig cuttings had a smaller fresh weight than the leaves of the healthy leafy-twig cuttings and yet produced the heavier root systems. The grams of fresh roots produced per gram of fresh leaves were as follows: healthy dipped, 0.272; healthy control, 0.277; mottled dipped, 0.403; mottled control, 0.369.

Lemon cuttings were also studied similarly. The leaf cuttings were grown from February 22 to April 17, 1935, at which time the mottling had disappeared from practically all of the leaves. The data obtained are presented in table I.

The zinc appears to have increased the rooting of healthy but not of the mottled leaf cuttings. The fresh weight of average root systems was 33 per cent of the fresh weight of an average healthy zinc-treated lemon leaf cutting and 38 per cent of the fresh weight of an average mottled zinc-treated leaf cutting. The relation of the fresh weight of roots produced to the weight of the zinc-treated leaf cuttings was therefore approximately the same for healthy and mottled leaves.

In table I are given also the data obtained in the rooting of lemon leafy-twig cuttings. A larger percentage of leaves of the healthy than of the mottled leafy-twig cuttings died. An average leaf of the mottled leafy-twig cuttings was only about half the fresh weight of an average leaf of the healthy leafy-twig cuttings of lemon. A slightly greater percentage of the leaves of treated than of untreated leafy-twig cuttings died. All of the mottled leafy-twig cuttings of lemon rooted while 90 to 92 per cent of the healthy ones rooted.

The grams of fresh roots per gram of fresh leaves of the leafy-twig cuttings were determined: healthy treated, 0.293; healthy control, 0.344; mottled treated, 0.278; mottled control, 0.430. These results (if the twigs are not taken into consideration) indicate a retardation

of root growth when the leaves of the healthy and mottled lemon leafy-twig cuttings were zinc-treated, and show that the leaves of the control mottled cuttings were associated with a greater root production than were those of the control healthy cuttings.

The grams of fresh roots produced per gram of fresh twig (without leaves) of the leafy-twig cuttings of lemon were: healthy treated, 0.905; healthy control, 1.061; mottled treated, 0.884; mottled control, 1.155. These results for twigs and roots bear the same relation as those for leaves and roots.

EFFECT OF ENVIRONMENTAL FACTORS.—During the course of these experiments the observation (3, p. 541) was repeatedly made, regarding the increase in green color of mottled leaves, that during the rooting process the mottled leaves in many cases became dark green and healthy in appearance, although the shape of the severely mottled leaves underwent no change. At least three factors may operate to bring about this recovery process: (1) the reduction in the intensity and the change in the quality of the light may affect the cuttings grown in the rooting chamber; (2) the degree of temperature (about 28° C.) and the relative humidity of the rooting chamber in conjunction with the reduced and altered light may favor vegetative growth; (3) the cuttings may absorb appreciable zinc from the river gravel in the rooting chamber.

Citrus trees when grown in the field under cloth or indoors under glass without direct sunlight produce luxuriant, tender, green, vegetative growth. The south-exposed portion of the trees in the field are often the most severely mottled. The duration of period of exposure to, and intensity of, sunlight therefore appear to be associated with mottle-leaf.

Severely mottled cuttings of orange and lemon recover partially or fully their green color when planted in gravel in rooting chambers regardless of whether the leaves are dipped in water, in a mixture of hydrated lime, in blood albumin spreader, or in this mixture plus zinc. Light, temperature, and humidity under these conditions are factors favoring a healthy appearance. In the field, leaves of the late autumn cycle that mottle as lower temperatures prevail may become fully green in the spring upon the return of higher temperatures.

The leaves of leafy-twig cuttings of lemon grown in a culture solution to which no zinc was added, except as impurities, became yellow near the tip and tipburned when grown under continuous illumination. Some of the leaves showed a suggestion of mottle.

Various mottle patterns were found in the leaves when leafy-twig cuttings of Valencia orange were grown in culture solutions in much used, 4-liter capacity, flint glass battery jars each containing three cuttings and with no zinc added except as impurities. The culture solutions were changed only at long intervals and the cultures were subjected to strong continuous illumination. Mottle also occurred under these conditions when 1 p.p.m. of zinc was added to the culture solution, but not when larger concentrations were used. Intense light no doubt can increase the requirements of a plant for a given element such as zinc. Mottle-leaf of citrus frequently is most severe in the bright hot interior districts of southern California.

In order to eliminate the possibility of the absorption of zinc and other materials from the river gravel as factors in the recovery from mottling while in the rooting chambers, experiments were conducted using pure Ottawa silica sand. Such sand was repeatedly acid-treated and washed with distilled water and placed in acid-washed glass or Swedish enamelware pans. Severely mottled leafy-twig cuttings of lemon were placed in the sand. These sand cultures were then stored in the rooting chambers and within a few weeks the leaves showed a considerable increase in green color and the mottling became inconspicuous.

GERICKE (2) has shown that wheat plants grown in solution cultures devoid of iron remain green when grown in light of low intensity and produce excellent growth; whereas when the cultures are devoid of iron and are exposed to bright sunlight, the plants make little growth and become markedly etiolated. It is known that while light acts as a catalyst in chlorophyll formation, it also acts as a destroyer of chlorophyll, so that the net effect of these two processes becomes of importance. Light, temperature, and drainage effects may not be ignored as factors in studies of the effect of zinc on mottle-leaf, especially in cases of spontaneous recovery of trees from mottle-leaf when grown in soils.

In citrus the last cycle of growth which frequently matures during

the cold weather of autumn is the most likely to mottle. The effects of temperature on the solubility of soil constituents, on the absorption by the roots, and on photosynthesis, are of importance in the production of mottle-leaf and in the maintenance of an adequate supply of zinc and other constituents.

In walnuts it is usually the first cycle of growth that tends to mottle. Unless migration of zinc and other elements back into the twigs takes place, in any given season the abscission of the leaves in the late autumn may reduce the supply of zinc and other constituents available for the rapid development of the first cycle in the following spring. The rate at which traces of elements are available in relation to the rapidity of growth is an important consideration.

Sugar content of leafy-twig cuttings of Valencia orange in relation to mottling

The environmental conditions in the rooting chamber were similar for all the cuttings. No new top growth was allowed to develop. While in effect this may be considered as a form of pruning, it was carried on similarly in all cuttings. Only the growth that took place in the roots was allowed to remain.

At the end of the experiments the various portions of the different lots of leafy-twig cuttings were rapidly washed with distilled water, dried between filter paper and wiped with diaper cloth. The plant fractions were then rapidly dried below 65° C., after which they were ground in a Wiley mill.

The data for the sugar determinations (table II) suggest that the zinc treatment lowered the percentages of reducing and total sugars. A large percentage of total sugars, chiefly sucrose, was found in the roots. The sugar content of healthy and of mottled citrus leaves was further investigated because it has been shown that mottled leaves are richer in nitrogen, phosphorus, and potassium but lower in calcium than healthy leaves; and because frequently in the field it is observed that, unless in too advanced a stage of mottling, mottled trees (for a given size of the top) may show no appreciable decreased fruit production below that of healthy trees of the same size. It is not known how severe the mottle-leaf condition must be in order to affect the yield.

Further data were obtained regarding the sugar content of healthy and of mottled citrus leaves. The samples were collected sometimes from single trees or sometimes from numerous trees. In each case the healthy and mottled samples were obtained from the same trees at approximately the same time. This procedure was followed because rarely are healthy trees found that do not bear some mottled leaves.

TABLE II

PERCENTAGE REDUCING AND TOTAL (AS REDUCING) SUGARS IN DRY MATTER OF LEAVES, TWIGS, AND ROOTS OF VALENCIA ORANGE LEAFY-TWIG CUTTINGS

HEALTHY CUTTINGS				MOTTLED CUTTINGS			
DIPPED IN ZINC MIXTURE		NO ZINC (CONTROL)		DIPPED IN ZINC MIXTURE		NO ZINC (CONTROL)	
REDUCING SUGARS	TOTAL AS REDUCING SUGARS	REDUCING SUGARS	TOTAL AS REDUCING SUGARS	REDUCING SUGARS	TOTAL AS REDUCING SUGARS	REDUCING SUGARS	TOTAL AS REDUCING SUGARS
Leaves							
1.62.....	2.88	1.70	3.18	1.74	3.58	1.86	4.77
Twigs							
1.32.....	3.05	1.78	3.17	1.45	3.72	1.80	3.73
Roots							
1.49.....	6.35	1.47	6.51	1.71	6.63	2.75	8.08

The data for the reducing and the total (as reducing) sugars are given in table III. The dry matter of mottled leaves contains slightly greater percentages of reducing and total sugars than that of healthy leaves; such higher content may well account for increases in the percentage of rooting, for the total amount of roots produced in cuttings, and for the apparently quicker response of mottled over that of healthy trees to applications of zinc in the field.

ARTIFICIAL PRODUCTION OF MOTTLE-LEAF IN SOIL AND SAND CULTURES

It was difficult to produce mottle-leaf out of doors when the trees were grown in sand cultures in large galvanized iron containers.

This may have been due to penetration by the root of the asphalt paint on the inside of the container whereby the trees obtained a supply of zinc. The closest approach to mottle-leaf as it occurs in the field was produced in well drained sand or soil cultures in asphalt-coated zinc cans, in earthenware pots, or in wooden containers when

TABLE III

PERCENTAGE REDUCING AND TOTAL (AS REDUCING) SUGARS IN DRY MATTER OF CITRUS LEAVES

Variety	Collected	Location	Reducing sugars		Total as reducing sugars	
			Leaves not mottled	Leaves mottled	Leaves not mottled	Leaves mottled
Navel orange on sour orange stock	Dec. 11, 1930	Pathology plots, R6, T5, 6, 7	2.20	2.58	4.14	4.40
Marsh grapefruit on sweet orange stock	Dec. 11, 1930	Pathology plots, R18, T3	2.29	2.74	3.74	5.60
Valencia orange on sweet orange stock	Jan. 9, 1933	Plot A, Rubidoux	1.95	2.10	2.96	3.04
	Jan. 12, 1933	Plot G, Rubidoux	1.80	2.09	4.46	4.20
	Jan. 12, 1933	Plot H, Rubidoux	1.75	2.13	5.90	5.90
	Feb. 3, 1933	Plot K, Rubidoux	1.95	2.74	5.76	6.42
	Feb. 17, 1933	Plot S, Rubidoux	1.82	2.19	8.14	7.48
	Feb. 18, 1933	Plot V, Rubidoux	2.17	2.32	3.56	3.66

the cultures received a solution containing urea, arsenic, cyanamid, or dicyanamid (figs. 2, 3). Calcium nitrate was usually added in conjunction with these materials, especially when they were used in high concentrations. Calcium nitrate alone failed to produce the symptoms in the leaf. When the substances were used without calcium nitrate the injury was often sudden and severe. The addition

of calcium nitrate usually was accompanied by an increase in growth which lessened the injury and tended to show more of the intermediate stages.

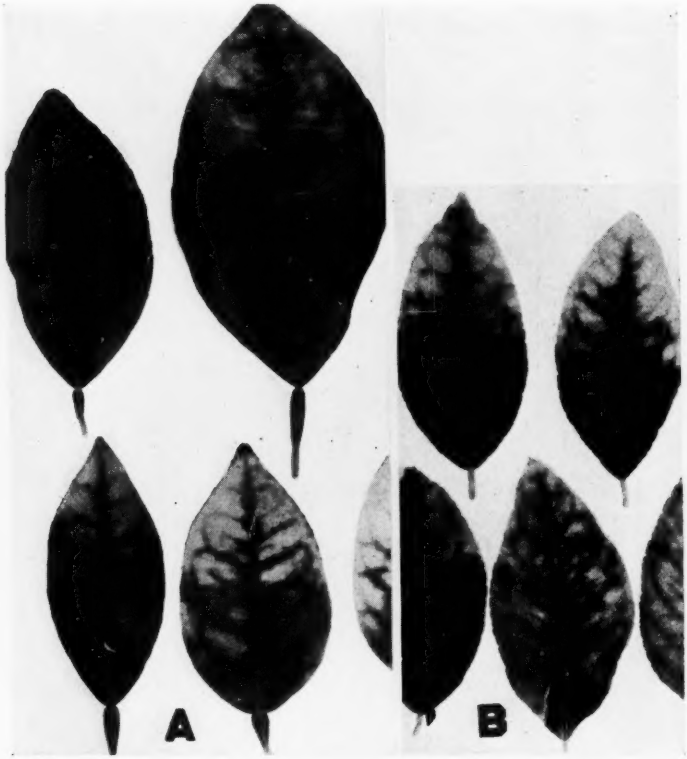

FIG. 2.—Type of mottle in leaves from trees grown in either Sierra loam soil or sand cultures in galvanized iron containers, glazed crocks, or boxes, with addition of urea-calcium nitrate mixture to the soil or sand: *A*, Valencia orange (upper left, leaf from control culture); *B*, lemon.

Some of the symptoms of mottling produced in artificial cultures may not be considered by some investigators as being the "true" mottle-leaf as seen in the field, although there are many different so-called types of mottling that extend over a rather wide range of

patterns. A mottled condition exists when the chlorosis does not involve the veins. One criterion as to whether a chlorosis is a true mottle is the degree of recovery brought about by the use of zinc.

In the artificial production of mottle-leaf, difficulty was encountered in supplying sufficient iron to prevent complete chlorosis because of the tendency toward alkalinity and in preventing in the presence of considerable calcium in the urea mixture the appearance of symptoms of potassium and boron deficiency. The use of urea or

FIG. 3.—Various types of mottle produced in citrus leaves: *A*, left, grapefruit leaf from leafy-twig cuttings grown in solution or in Sierra loam soil cultures containing excessive arsenic; right, Valencia orange leaf from trees grown in pot cultures with soil containing excessive arsenic in any of several forms. *B*, effect of excessive cyanamid or dicyanamid on lemon leaves from budded trees in soil cultures in galvanized iron containers.

a urea-calcium nitrate mixture, cyanamid, dicyanamid, or arsenic was accompanied by varying degrees of partial chlorosis, depending on the amount and frequency of adding the materials to the soil. Large amounts, particularly of the urea-calcium nitrate mixture, brought about severe symptoms, including little-leaf. By regulating the amounts applied, the frequency of their addition, and the extent of drainage, the appearance of symptoms of chlorosis in the new growth could be controlled. As the nitrification of the urea to nitrate progresses, the new leaves produced are healthy and the affected leaves show some improvement. Similarly in soil cultures in wooden containers or in galvanized iron tanks as large as 8 feet in diameter and 4 feet deep, typical little-leaf and a mottled type of

chlorosis could be brought about, not only in citrus but also in walnuts (fig. 4), by controlling the application of urea-calcium nitrate mixture.

Calcium nitrate alone showed no injurious effect. The severity of the disease could be controlled as previously described for citrus.

FIG. 4.—Walnut leaflets from trees grown in Sierra loam soil in galvanized iron tanks: Left, control; remaining leaflets show varying degrees of chlorosis between the veins. Symptoms resulted from use of various urea-calcium nitrate mixtures or urea alone but not calcium nitrate alone. Reduction in size of leaflets and severity of mottle could be controlled by the time and amount of application.

There is one point of similarity between the artificial production of the type of mottle in walnut leaves in soil cultures and that which occurs in the field, in that under artificial conditions the mottle was always produced on the first cycles of growth but never on the late cycles. In the field it is the first cycle of growth that is by far the most severely affected; in many cases the later cycles of growth appear healthy.

Since urea and many of the other compounds employed contain the NH_2 group, it is possible that a mottle type of chlorosis may be associated with the nitrogen group when the absorption of large amounts of such incompletely nitrified constituents takes place. Zinc is an element known especially for its oxidation-reduction activity and for its catalytic functions. The zinc absorbed from the soil and container appears to be adequate when only small amounts of the urea mixture are applied, but with large amounts the zinc absorption possibly cannot keep pace with the absorption of these incompletely nitrified constituents. Further studies of the relation of absorbed to assimilated nitrogen groups are to be undertaken.

The use of $NaNO_3$ in soil cultures without drainage was associated with increasing alkalinity and mottling in citrus, and especially in pecans. The alkalinity may have greatly reduced the concentration of soluble zinc and other elements essential for healthy growth; while the excessive nitrate, before injury was manifest, further reduced the relative amounts of these constituents in the tissues by the dilution factor brought about by the stimulation of active vegetative growth.

When the leaves of orange trees grown in cultures containing urea were dipped in a zinc-lime-spreader mixture the mottle became less pronounced, although the time allowed for the zinc to act also gave the nitrification processes in the soil more time in which to transform the urea more completely to the nitrate stage, so that the tests lacked adequate control.

VAILE (10) has pointed out that the use of nitrogen in the form of nitrate of soda and to a less degree in the form of dried blood, without the use of bulky organic manure of some sort, is conducive to the development of mottle-leaf in citrus trees. Leaves of trees on plots without nitrogen additions showed practically no mottling. These deductions may have been based on the fact that trees which receive no nitrogen make comparatively little new growth, and the requirement under such conditions for zinc and other constituents is extremely small compared with the requirements when relatively pure nitrogen fertilizer additions have induced marked vegetative growth. Bulky organic manures on the other hand contain fairly large amounts of a considerable number of constituents other than nitrogen, among which is zinc.

RELATION OF ZINC TO PRESENCE OF MOTTLE-LEAF IN EARLY STAGES OF GROWTH IN SOLUTION CULTURES

It was shown by HAAS (3) with lemon cultures that zinc in minute amounts in the culture solution was of benefit to the quality of the growth, while somewhat greater amounts were distinctly injurious.

Some of the factors that have retarded the drawing of conclusions on the effect of zinc on citrus are the minute amounts necessary for growth, the difficulty of preventing zinc contamination, the need of chemical methods for determining minute amounts (5), and the absence of an adequate procedure for growing citrus in solution cultures.

Until recently, leafy-twig cuttings of orange could not be grown in culture solutions on their own roots beyond a certain stage. Considerable success, however, was had in growing such cuttings when they were used as scions on lemon or rough lemon leafy-twig cuttings as stocks, and the more so as the phosphorus nutrition in relation to that of the minor elements was better understood.

In order better to determine the causal factors involved in mottle-leaf, it is essential that mottling be produced in solution cultures. Although no zinc was added to solution cultures (except as impurities in the so-called "chemically pure" salts), mottling thus far has not been produced with lemon or rough lemon leafy-twig cuttings alone, or with leafy-twig cuttings of orange as scions on lemon or rough lemon leafy-twig cuttings as stocks. This emphasizes the importance of the results of LEE (7), who found that a given variety may differ in its susceptibility to mottle according to the stock on which it is grown.

In order to test the effectiveness of zinc upon growth and upon the incidence of mottle-leaf, unaerated solution cultures were conducted with rooted leafy-twig cuttings of Valencia orange in Swedish enamelware shallow pans. The cuttings were placed in the cultures on October 19, 1934 and were photographed on April 25, 1935. The culture solution employed (4) was that previously used by HOAGLAND, and contained the single strength calcium nitrate with iron, boron, and manganese added as minor elements. Phosphate was present in the solution for only a few days at intervals of several weeks. When it was absent from the solution, then aluminum or

zinc or both were added. The pH values of the culture solutions were raised to approximately 6 by means of a solution of calcium hydroxide.

Healthy growth was obtained when either 0.5 p.p.m. zinc or 0.5 p.p.m. aluminum or both were present in the culture solution. Increased growth, however, was obtained when 1 p.p.m. zinc or 1 p.p.m. aluminum or both were present. These approximate concentrations furnish a basis for subsequent studies relative to zinc in culture solution.

At higher concentrations of zinc, as HAAS (3) has previously pointed out, injurious symptoms of zinc poisoning occur. In figure 5*B* it is seen that when 5 p.p.m. of zinc were present the smallest amount of growth resulted. The roots at first appeared healthy but soon showed injury. The use of 5 p.p.m. aluminum was accompanied by excellent growth when zinc was not added. Addition of aluminum resulted in improved growth when the culture solution contained an excess of zinc.

Control cultures were conducted in which neither zinc nor aluminum was added to the solution. Figure 5*A*, left, shows the growth made by such cultures. The leaves were quite yellow. Figure 5*A*, right, shows another such culture but which received 5 p.p.m. aluminum several months after the culture was started. The root growth was increased and the leaves were dark green.

In every case the use of zinc brought about a dark green color in the leaves without the appearance of mottling. It is of interest that when aluminum was used without zinc (fig. 5*A*), mottling occurred even though the leaves of the original cuttings were dark green and healthy in appearance (fig. 6*A*). It should be emphasized that this is the first instance of the artificial production of mottle-leaf in controlled solution cultures and forms a basis for further investigations on the zinc relation in mottle-leaf. The omission of zinc from the culture solution was not accompanied in every case by mottling, and it remains for future studies with more highly purified solutions to ascertain the reasons.

Badly mottled leafy-twig cuttings of Valencia orange are shown in figure 6*B*. The solution contained 5 p.p.m. of aluminum but no added zinc. The mottle disappeared when zinc was added.

In order to study the effect of coating leaves with zinc mixture, rooted leafy-twig cuttings of Valencia orange were grown in a culture solution to which no zinc was added. Some of the tops were dipped

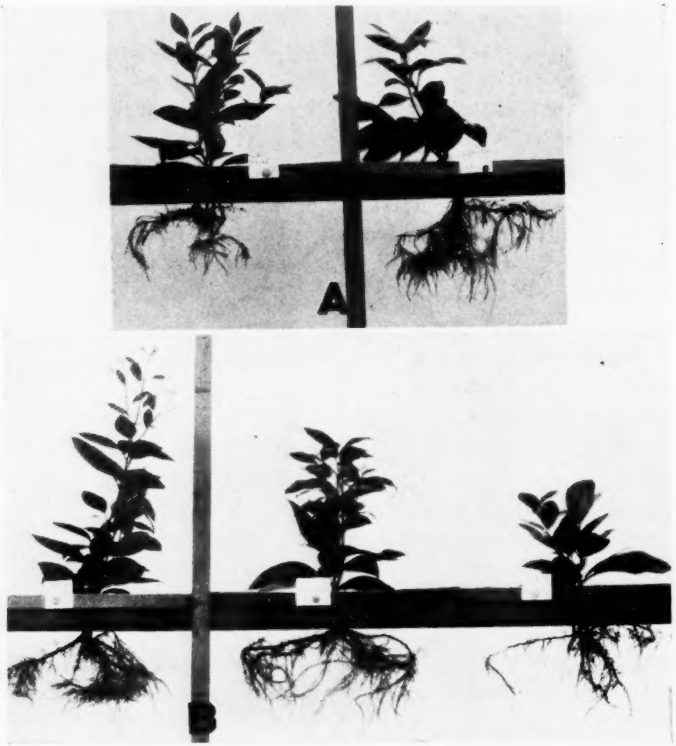

FIG. 5.—Leafy-twig cuttings of Valencia orange grown in culture solution: *A*, left, without zinc or aluminum added; right, without zinc but with 5 p.p.m. aluminum added several months after culture was started. *B*, left, 5 p.p.m. aluminum; right, 5 p.p.m. zinc; center, 5 p.p.m. zinc and 5 p.p.m. aluminum.

in the mixture, while the leaves of some cuttings were coated on the upper side and those of others were coated on the lower side.

The tops of cultures (fig. 7*A*) were dipped in the zinc mixture while the leaves of others (fig. 7*B*) were painted with the mixture

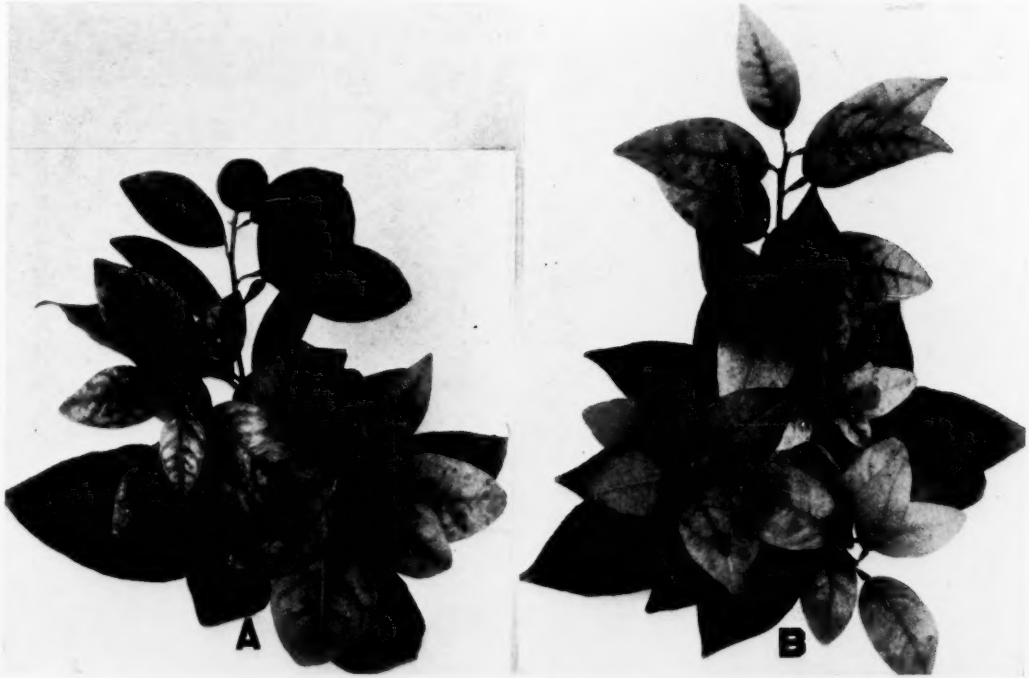

Fig. 6.—Severe mottling of Valencia orange leafy-twig cuttings in culture solution: *A*, same as figure 5*A* but photographed to show mottling of new leaves. Note dark green, healthy leaves of original cuttings. *B*, 5 p.p.m. aluminum, no zinc; recovery followed addition of zinc.

on the upper surface only. The cultures were grown from December 28, 1934 to April 25, 1935. At the end of this period the cuttings of which the leaves were coated only on the upper surface made the greatest initial growth. When the zinc coating was applied to both the upper and lower surfaces, growth was retarded.

Subsequently these cuttings made healthy growth, surpassing in quantity and quality that of cuttings not apparently checked. Earlier in this paper it was shown that the zinc treatment retarded the root growth of a lot of lemon leafy-twig cuttings. Such temporary or transient toxic effects of zinc absorption, as well as the state of

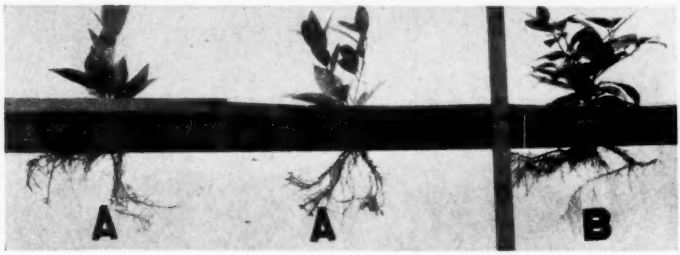

FIG. 7.—Effect on growth of coating surfaces of leaves of solution cultures of Valencia orange leafy-twig cuttings with mixture of zinc. *A*, leaves dipped in mixture; *B*, only upper surface of leaves coated.

the nutrient reserves in the tree, possibly may account in part for the marked variations that frequently are noted in the response of trees to zinc sprays.

Summary

1. With both healthy and mottled Valencia leaves the zinc treatment increased the number of leaves that rooted. Twenty-eight per cent of the zinc-treated healthy Valencia leaves and 20 per cent of the control (not treated) healthy leaves rooted. Thirty per cent of the zinc-treated mottled Valencia leaves and 6 per cent of the control (not treated) mottled leaves rooted. Fewer leaves died among the zinc-treated than among the control leaves.

2. Although the average fresh weights of the mottled orange leaves are less than those of the healthy leaves, the average fresh

weights of the root systems of the mottled leaves are greater than those of the corresponding healthy leaves.

3. Of the healthy lemon leaf cuttings dipped in zinc mixture, 52 per cent rooted; of those not dipped (control), only 26 per cent rooted. Zinc treatment benefited the rooting of healthy leaves but failed to improve the rooting of mottled lemon leaves. Over 60 per cent of mottled lemon leaf cuttings rooted regardless of whether zinc treated or not. Mottled lemon leaf cuttings root as readily if not better than healthy lemon leaves.

4. The leaves of mottled Valencia leafy-twig cuttings had a smaller fresh weight than the leaves of healthy leafy-twig cuttings, and yet produced the heavier root systems. The treatment of mottled Valencia leafy-twig cuttings with zinc mixture increased the grams of fresh roots per gram of fresh leaves.

5. The zinc treatment retarded the root growth of lemon leafy-twig cuttings. The untreated mottled lemon leafy-twig cuttings produced a greater fresh weight of roots than the untreated healthy lemon leafy-twig cuttings.

6. The various portions of untreated (control) rooted mottled Valencia leafy-twig cuttings contained greater percentages of sucrose than the corresponding portions of healthy (not mottled) cuttings. The zinc treatment lowered the percentages of sucrose present in the leaves and roots at the end of the experiment.

7. The dry matter of mottled leaves grown in the field contains slightly greater percentages of reducing and total sugars than does that of healthy leaves. This increased sugar content is of advantage in bringing about a rapid recovery once the limiting condition has been corrected.

8. In sand or soil cultures, an excess of urea, urea-calcium nitrate mixtures, cyanamid, dicyanamid, or arsenic brought about various stages of partial chlorosis. In the case of urea, little-leaf was induced, the severity of which could be controlled by regulating the amount and frequency of the applications.

9. Rooted cuttings grow well in solution cultures when phosphate is supplied for a few days at intervals of several weeks and when aluminum is present during the absence of phosphate.

10. Mottle-leaf was produced for the first time in Valencia leaves

of rooted leafy-twig cuttings grown in culture solutions. In solution cultures mottling has been produced thus far only in the absence of zinc or in the presence of low zinc concentrations but under continuous high light intensities.

11. When zinc concentrations in the culture solution are somewhat excessive, the addition of aluminum gave beneficial effects on growth. The use of zinc in the culture solution in not too high a concentration was followed by a dark green color in the leaves.

12. By means of coating with a mixture of zinc the surfaces of leaves of rooted Valencia leafy-twig cuttings grown in culture solutions, it was possible to note the toxic effect of zinc or its temporary inhibiting or retarding effect on growth. Such effects may partially account for the variations that frequently are observed in the rate of response of trees to zinc applications.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

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EFFECT OF ATMOSPHERIC HUMIDITY ON RATE OF CARBON FIXATION BY PLANTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 473

JOHN W. MITCHELL

(WITH FOUR FIGURES)

Introduction

That the synthesis of solid materials by many kinds of plants is inhibited when the surrounding air is relatively dry has been shown by several investigators (**5, 10, 15, 18**). LEBENDINCEV (**12**) and others have substantiated the view that a decrease in the water content of leaves is generally accompanied by a decrease in the rate of carbon fixation (**2, 3, 4, 8**).

The purpose of the present investigation was to determine whether any direct correlation between atmospheric moisture, rate of photosynthesis, and accumulation of dry weight by certain plants could be established. Leaves and also the entire tops of plants inclosed in glass chambers were supplied with air streams containing different known amounts of moisture, and the rate of carbon fixation measured. All environmental factors were controlled and maintained constant with the exception of those under consideration. The stomatal behavior and to some extent the quantitative carbohydrate content of leaves grown at different humidities were determined.

Methods

Plants were grown in a greenhouse in fertile soil contained in clay pots. They were placed in a room in which environmental factors were controlled, and supplied with artificial light during the measurement of carbon fixation rates (**17**). The soil was kept continually moist by placing the pots in shallow dishes that contained water about 1 cm. deep.

Methods and apparatus used in estimating the rate of carbon fixation by leaves have been described (**17**). These methods, how-

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

ever, were modified for estimating the rate of carbon fixation of the above-ground portion of plants. This modification consisted of encircling the stem of a plant with the two halves of a circular metal plate which had an opening in the center for the stem (fig. 1). The two metal pieces were held together with screws, the union sealed with a mixture of clay and castor oil, and the space between the

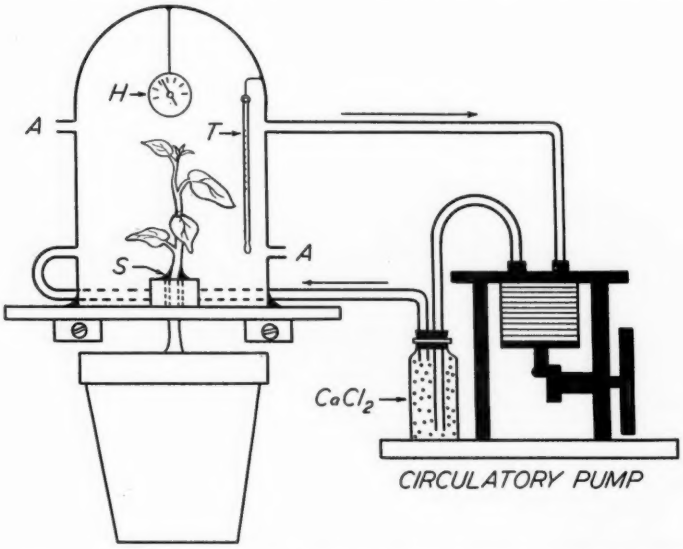

FIG. 1.—Apparatus used to study effect of relative humidity on rates of carbon fixation by plants: *A*, *A*, inlet and outlet for air stream; *T*, thermometer; *H*, hygrometer; *S*, clay seal.

stem and metal also sealed in the same manner. A glass chamber with suitable outlets was then placed over the part of the plant extending above the plate, and an airtight union was made between the glass and metal by means of the oil and clay mixture. A circulatory pump, attached by means of rubber tubing to outlets in the chamber, recirculated the inclosed gas through the pump and chamber at a rate of 130–180 liters per hour. By means of other outlets in the chamber (*A*, *A*), air slightly enriched with CO_2 was passed through at a rate of 15.6 liters per hour, and the absorption of CO_2

by the plant was determined by means of an electrical conductivity method (**17**).

The humidity of the air inclosed by this type of chamber was controlled by the use of a drying agent through which the air was circulated (fig. 1). Moisture was removed from air surrounding the inclosed leaves in the same manner. The relative humidity of the atmosphere was indicated by hygrometers which were suspended inside the chambers directly above the inclosed plants (*H*). These instruments were calibrated by suspending them in a bottle above solutions of sulphuric acid and water of various known concentrations. During the calibration, the bottle containing the hygrometer

TABLE I

CALIBRATION OF HYGROMETER BY MEANS OF KNOWN HUMIDITIES PRODUCED OVER SOLUTIONS OF SULPHURIC ACID

PERCENTAGE OF ACID	TEMPERATURE (C°.)	SPECIFIC GRAVITY	RELATIVE HUMIDITY	READING
17.01	20.0	1.12	92.3	90.0
41.60	20.0	1.32	57.3	54.0
61.0	20.0	1.51	17.0	18.0
79.0	20.0	1.72	2.7	0.0

and acid was closed with a stopper through which were inserted a thermometer and inlet and outlet tubes to which a circulatory pump was connected and used to circulate the inclosed air. Various known humidities were produced in this manner (table I) and curves were plotted by means of which the hygrometer readings were corrected.

In determining the effect of atmospheric moisture on carbon fixation rates, the inclosed leaves or tops of plants were first exposed to moist air, then to relatively dry air, maintaining other environmental factors constant while the rate of carbon fixation was measured. Granular $CaCl_2$ proved most suitable for removing moisture from the air. In some cases, however, solutions of sulphuric acid diluted with different amounts of water were used as drying agents with no apparent injury to the plants.

The method of carbohydrate analyses referred to later consisted in digesting the dried material in 2.5 per cent HCl for two and one half hours under a reflux condenser. The solution was cooled and

made to volume. It was then filtered and the filtrate neutralized with anhydrous sodium carbonate. Reducing sugars were then determined (**19**) and calculated as glucose.

Experimental results and discussion

Recent papers (**6, 10, 11**) show that the rates of carbon fixation by many kinds of plants fluctuate during a given period of illumination under natural conditions. It was desirable to determine whether similar changes occurred in the rates of carbon fixation by plants grown in the constant environmental conditions used in these experiments. The rates at which carbon was fixed by attached leaves of *Lycopersicum esculentum* (Mill.), *Pelargonium zonale*, *Senecio cruentus* (DC), *Phaseolus vulgaris* (Linn.), and *Primula obconica* (Hance) under a constant environment were determined hourly over periods of 3–8 hours for intervals varying from two to fourteen days. The intensity of artificial light to which the leaves were exposed was constant for any individual experiment, but varied between 900 and 1200 foot candles between different experiments as measured with a Weston light meter. The temperature, humidity, and rate of circulation of air around the leaves were maintained constant, while variations in the concentration of carbon dioxide in air surrounding the leaves was maintained within the range of fluctuation found in the concentrations of CO_2 of air under natural conditions. The amounts of carbon fixed by the leaves of the plants mentioned varied slightly from hour to hour, during a given period of illumination. Data obtained from experiments with tomato leaves showed that the maximum deviation from the mean rate of carbon fixation exhibited by an individual leaf over a period of seven hours was approximately 14 per cent (table II). The hourly variations in the rates of carbon fixation of the leaves of other plants was generally less and seldom greater than this amount.

Effect of humidity on rate of carbon fixation

Six plants of *Primula obconica* were selected from a number grown in a greenhouse during the fall and winter and placed under artificially controlled conditions. One leaf on each plant was chosen with respect to size, position on plant, and color, and after 25 hours

these attached leaves were inclosed in individual chambers. Air at a temperature of 24.5–25.5° C. was enriched with CO_2 such that on leaving the chambers it contained this gas in amounts within the range of fluctuation found under natural conditions. The enriched air was drawn at the same rate through each chamber for two hours. During this time the moisture content of air surrounding the inclosed leaves increased to 70–80 per cent of saturation, due principally to transpiration. The amounts of CO_2 absorbed by the individual leaves when surrounded by air of this moisture content were meas-

TABLE II

CARBON DIOXIDE ABSORBED BY TOMATO LEAVES UNDER CONSTANT CONDITIONS. RESULTS CALCULATED AS MILLIGRAMS PER HOUR PER HUNDRED SQUARE CENTIMETERS OF LEAF SURFACE

No. of leaf	Hour						
	1st	2nd	3rd	4th	5th	6th	7th
9	17.9	14.4	17.5	17.3	14.5	18.0	17.4
11	19.0	20.1	19.4	20.0	20.6	17.8	20.7
13	16.1	17.9	17.1	19.6	16.0	17.2	19.3
14	16.7	17.9	17.5	18.8	16.0	17.2	17.9
15	16.1	17.9	17.1	16.6	16.1	16.3	18.9
16	17.0	15.2	17.6	16.2	16.2	16.4	17.2
Average	17.1	17.2	17.7	18.1	16.5	17.2	18.6

ured during the third hour (table III). Flasks containing 20 per cent sulphuric acid were then connected between the circulatory pumps and plant chambers as previously described. The chambers were again flushed for two hours with enriched air, during which time the moisture content of the inclosed air decreased to 40–50 per cent of saturation. The amounts of CO_2 absorbed by the inclosed leaves at this humidity were determined during the third hour. The humidity of air in the chambers was reduced still farther by the use of 50 per cent solutions of sulphuric acid, and the procedure of flushing repeated. The amount of CO_2 absorbed by the inclosed leaves at a humidity of 10–20 per cent was determined. The leaves appeared turgid during exposure to low humidities and showed no evidence of wilting, although careful examinations were made immediately after

they were removed from the chambers. The plants were returned to the greenhouse. No evidence of injury occurred during the period of experimentation nor during the following three weeks.

The preceding experiment was repeated using attached leaves of bean, tomato, *Pelargonium*, *Brassica oleracea* (Linn.), and *Cucurbita pepo*. Dry $CaCl_2$ and solutions of sulphuric acid were used to remove moisture from the air in the chambers. Results obtained with the leaves of these plants were similar to those obtained with the leaves

TABLE III

CARBON DIOXIDE ABSORBED BY LEAVES OF PRIMULA WHEN SUBJECTED TO DIFFERENT HUMIDITIES. RESULTS CALCULATED AS MILLIGRAMS PER HOUR PER HUNDRED SQUARE CENTIMETERS OF LEAF SURFACE

No. of leaf	Percentage humidity		
	70–80	40–50	10–20
38	14.0	10.9	11.9
39	10.8	10.0	11.3
40	16.0	15.0	12.6
41	12.1	14.0	13.0
42	12.4	12.0	12.3
43	16.7	17.0	16.0
Average	13.7	13.2	12.9

of *Primula* and in no case did the inclosed leaves wilt, exhibit injury, or show a significant reduction in the rate of carbon fixation when exposed for three-hour intervals to low humidities.

EFFECT OF WILTING ON RATE OF CARBON FIXATION

Six plants of *Cineraria* were selected from a number which had grown in a greenhouse during the fall and winter. One leaf attached to each plant was chosen with respect to size, position on stem, and color, in order to have them as nearly uniform as possible. The plants were placed under controlled environmental conditions and after 24 hours the selected leaves were inclosed in leaf chambers. Enriched air at 24.5–25.5° C. was drawn through the chambers and the amount of CO_2 absorbed by the inclosed leaves determined during the third hour, as in previous experiments. No significant differ-

ences were noted in the rates of carbon fixation when the leaves were surrounded for three hours with air having a relative humidity of 70–80, 50–60, or 20–30 per cent (table IV). All the leaves remained turgid during the experiment and showed no evidence of injury after several weeks.

This experiment was repeated using older leaves of *Cineraria* taken from another group of plants and somewhat different results were obtained. Six leaves attached to individual plants were inclosed in leaf chambers after the plants had been under controlled environ-

TABLE IV

CARBON DIOXIDE ABSORBED BY LEAVES OF CINERARIA WHEN SUBJECTED TO DIFFERENT HUMIDITIES. RESULTS CALCULATED AS MILLIGRAMS PER HOUR PER HUNDRED SQUARE CENTIMETERS OF LEAF SURFACE

No. of leaf	Percentage humidity		
	70–80	40–50	10–20
1	14.9	15.1	16.2
4	11.0	9.0	12.0
6	13.8	13.8	13.0
18	16.0	14.6	16.1
21	14.2	13.8	13.3
23	13.1	12.9	15.0
Average	13.9	13.2	14.3

mental conditions for 24 hours. The chambers were flushed with enriched air as previously described, and the amount of CO_2 absorbed by the leaves in moist air was measured during the following three hours. The moisture content of the air in the chambers was then reduced by means of $CaCl_2$ and the amount of CO_2 absorbed by the leaves when surrounded by relatively dry air was measured during the following three hours. Three of the inclosed leaves became slightly wilted when surrounded with dry air, and these showed a decrease of 30–40 per cent in the rate of carbon fixation, while the other three remained turgid and absorbed approximately the same amount of CO_2 whether in dry or in moist air. Later the plants were placed in a greenhouse at a temperature of 55° F., and all of the

wilted leaves regained turgidity within two hours and showed no apparent signs during several weeks following the experiment of having been injured.

It is evident from the preceding experiments that individual leaves of squash, bean, cabbage, *Pelargonium*, and tomato plants remained turgid at a temperature of 25° C. although subjected to extremely low humidities. *Cineraria* leaves, which are especially sensitive to dry atmospheric conditions, in some few cases lost turgidity when the humidity of the surrounding air was rapidly decreased, and the loss of turgidity was accompanied by a marked decrease in the rate of carbon fixation. Further experiments concerning the effect of wilting on the rate of carbon fixation were not conducted, as other investigators (**2**, **3**, **4**) have shown that the desiccation of plants is often accompanied by a decreased rate of carbon fixation. It is emphasized, however, that low atmospheric humidity did not cause wilting in most of the plants studied. It is evident that in some cases dry air induces wilting only when accompanied by some other environmental condition, such as high temperature or low soil moisture, which also tend to reduce the water content of the plant.

Effect of increased temperature and prolonged exposure to low humidity on rates of carbon fixation

The leaves of *Pelargonium hortorum* were used in this experiment. The plants were 8–10 inches high and possessed about 15–25 leaves per plant. Six plants were selected from a group of several hundred and placed under the artificially controlled conditions previously described. The tops of the plants were inclosed in glass chambers (fig. 1) and the systems flushed with enriched air. The amount of CO_2 absorbed by each plant when the chambers contained moist air was then measured for a period of three hours. Air in the chambers was then dried with $CaCl_2$ and a relatively low moisture content was maintained for a period of 15 hours, while the soil around the roots was supplied with a surplus of moisture. Measurements made during the last three hours of the period showed an average increase of approximately 25 per cent in the amount of CO_2 absorbed per hour by the plants. During the experiment the plants appeared to

be equally as turgid as similar plants which were kept in the greenhouse. The leaves were immediately severed from the petioles,

TABLE V

WATER CONTENT OF PELARGONIUM LEAVES GROWN AT DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES

PLANT	PERCENTAGE RELATIVE HUMIDITY	TEMPERATURE (C.°)	VAPOR PRESSURE DEFICIT MM. HG.	PERCENTAGE LEAF MOISTURE
	AFTER 3-HOUR EXPOSURE TO HIGH HUMIDITY AND TEMPERATURE OF APPROXIMATELY 26° C.			
13	55–60	25.0–26.0	10.3	89.5
14	60–63	25.5–26.0	9.5	88.7
15	58–60	26.0–26.0	10.2	89.8
16	65–65	25.0–25.0	8.2	90.2
17	65–70	25.8–26.0	8.0	88.4
18	63–63	26.2–26.5	9.4	89.5
Average	62	25.8	9.3	89.4
	AFTER 15-HOUR EXPOSURE TO LOW HUMIDITY AND TEMPERATURE OF APPROXIMATELY 26° C.			
1	3–5	25.5–26.0	23.6	88.4
2	3–3	26.2–26.5	24.5	89.4
3	5–10	25.8–26.5	22.9	89.6
4	3–10	26.0–26.2	23.6	89.6
5	10–13	27.0–27.3	23.5	89.2
6	7–15	25.8–26.0	22.0	89.0
Average	7	26.2	23.4	89.2
	AFTER 3-HOUR EXPOSURE TO LOW HUMIDITY AND TEMPERATURE OF APPROXIMATELY 40° C.			
7	14–14	39.0–40.0	46.3	88.3
8	15–16	40.0–40.8	47.4	88.4
9	18–20	40.0–41.0	45.6	87.7
10	20–20	40.5–41.0	45.7	87.6
11	17–18	40.0–40.8	46.3	88.3
12	30–31	40.0–39.8	37.9	88.0
Average	19	40.2	44.9	88.1

weighed in weighing bottles, and after drying in a well aerated oven at 75° C., their moisture content was determined (table V).

BRILLIANT (1) has reported that the leaves of *Hedera helix* and *Impatiens parviflora* absorb carbon dioxide most abundantly when their water content is below maximum. The slight increase in the average amount of carbon fixed by the plants of the present experiments during prolonged exposures to dry air was not the result of desiccation, however, as the water content of the leaves remained approximately constant.

Results of this experiment show that the rates of carbon fixation by turgid leaves of silver-leaved *Pelargonium* were not reduced either

TABLE VI

AVERAGE AMOUNT OF CO_2 ABSORBED BY LEAVES OF PELARGONIUM UNDER VARIOUS CONDITIONS OF ATMOSPHERIC MOISTURE AND TEMPERATURE

RELATIVE HUMIDITY (10–30%)		RELATIVE HUMIDITY (60–80%)	
TEMPERATURE (C°.)	UPTAKE MG. PER PLANT PER HOUR	TEMPERATURE (C°.)	UPTAKE MG. PER PLANT PER HOUR
26	4.6	25	5.8
30	6.1	28	6.1
34	4.9	33	5.6
38	4.3	36	4.3
41	3.7	38	4.1

by a rapid decrease in the humidity of the air surrounding the plants nor by prolonged exposures of 15 hours to low humidity. This result seems logical, for although low humidity inhibits the vegetative growth of many kinds of plants, the accumulation of carbohydrates which often accompanies this response indicates that the process of photosynthesis is not greatly retarded (**5, 9, 15, 18, 20**).

In other experiments with silver-leaved *Pelargonium* the rate of carbon fixation was measured while the plants were surrounded by air at various temperatures and containing different amounts of moisture. A group of six plants was exposed to a relative humidity of 10–30 per cent. The temperature was then increased slowly from about 25° to approximately 40° C., during which time changes in the rate of carbon dioxide absorption were recorded. Another group of

six plants was subjected to a relative humidity of 60–80 per cent and a similar increase in air temperature, while variations in the rates of carbon dioxide uptake were measured (table VI).

The plants exposed to a gradually increasing temperature and low humidity showed an increased rate of carbon fixation, which reached a maximum at about 30° C. and then decreased as the plants

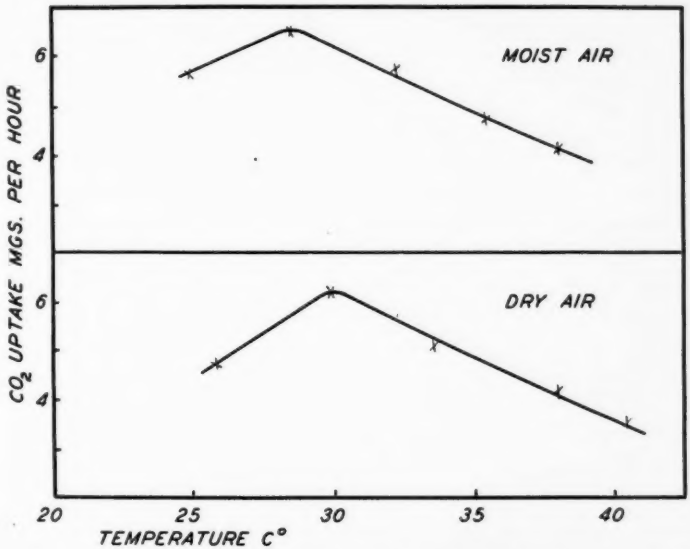

FIG. 2.—Graphs showing temperature at which maximum rate of carbon fixation occurred in group of *Pelargonium* plants subjected to high humidity (60–80 per cent) as compared with that of another group subjected to low humidity (10–30 per cent).

were subjected to higher temperatures. The rate of carbon fixation by similar plants which were subjected to the same temperatures, but surrounded with relatively moist air, also reached a maximum at approximately 30° C. and then decreased at higher temperatures (fig. 2). The moisture content of leaves subjected to relatively dry air and high temperatures was approximately the same as the moisture content of leaves under optimum conditions of temperature and humidity (table V). It is evident that such a decrease in the rate of carbon fixation was associated with high temperature rather than with desiccation of the leaves.

Effect of atmospheric moisture on stomatal aperture

In the course of the foregoing experiments, it was often noted that the stomata of the plants exposed to low atmospheric humidity were generally closed, even though the roots were abundantly supplied with moisture. Also statements based largely upon observations of plants growing under natural conditions indicate that when atmospheric humidity is high, stomata open wider and remain open longer than when it is low (**13, 15, 16**). It was decided, therefore, to study the situation in more actual detail under controlled environmental conditions when the relative humidity of the atmosphere was varied and the temperature maintained at 25° C. In none of the experiments was there an apparent loss of turgidity of the leaves, although the extent of the stomatal opening studied generally decreased with a decrease in the moisture content of the surrounding air, and the stomata finally appeared closed when the leaves were subjected to relatively dry air. The size of the stomatal openings was determined at different periods and at different relative humidities during the course of the experiments by direct microscopic observation of the surfaces of the leaves, by measuring the width of the openings by means of a micrometer ocular without removal of the epidermis from the leaf, and by a study of collodion membranes or molds made according to the method described by Long and Clements (**14**). Consistent results were obtained by all these methods.

As test plants the silver-leaved *Pelargonium* and tomato were used. The stomatal opening was measured as accurately as possible under the several conditions imposed. That carbon dioxide was fixed by the leaves was determined by three separate methods: (1) comparison of the amount of carbon dioxide absorbed by leaves under conditions in which the stomata were open and known to be favorable to photosynthesis with the amount absorbed by the same leaves when exposed to a dry atmosphere and with the stomata closed during the interval the photosynthetic measurements were made; (2) depleting the leaves of starch in the dark, as indicated by the iodine test, and then noting that an abundance of starch reappeared when the plants were illuminated in a relatively dry atmosphere even though the stomata were closed during the period of such exposure; (3) comparison, by means of chemical analyses, of the

carbohydrate content of leaves of plants illuminated in relatively dry air with the stomata apparently closed with the content of similar leaves in moist air having the stomata open.

Results from comparative methods

First method.—The amount of carbon dioxide absorbed by six plants when surrounded by moist air was first measured hourly for three hours. The relative humidity of the air around three of the plants was then reduced rapidly, while the moisture content of air

TABLE VII

Carbon dioxide absorbed by Pelargonium and tomato plants when first in moist and then in relatively dry air. Results expressed as milligrams of carbon dioxide absorbed per hour per plant

Hour	Percentage relative humidity			
	Tomato		Pelargonium	
	70–90	20–30	55–75	10–15
1st	8.1	7.5	3.9	4.3
2nd	7.5	7.7	4.5	4.1
3rd	8.1	7.2	4.6	4.6
Average	7.9	7.5	4.3	4.3

surrounding the remaining plants was unchanged. The amount of carbon dioxide absorbed during the following three hours under these conditions was approximately equal to the amount absorbed during the previous three-hour period when the plants were exposed to a more humid atmosphere (table VII). Following these measurements, the plants were removed individually and the stomata quickly observed microscopically. The time required for this observation was less than three minutes, during which period no appreciable change occurred in the size of the stomatal openings. The stomata of leaves on plants exposed to low humidities were apparently closed while those on the leaves of plants exposed to high humidities were open (table VIII, fig. 3). This experiment was repeated, using several

groups of tomato and geranium plants, with consistent results. In general the maximum size of stomatal opening occurred when the leaves were exposed to relative humidities above 60 per cent. The size of the openings decreased with a decrease in humidity, and the stomata generally appeared closed in leaves exposed to relative humidities below 30 per cent. It should be stressed, however, that gases such as carbon dioxide, oxygen, and water vapor may pass between the guard cells of stomata although they appear microscopically to be pressed tightly together.

TABLE VIII

SIZE OF STOMATAL OPENINGS IN LEAVES OF PELARGONIUM AND TOMATO PLANTS GROWN AT DIFFERENT ATMOSPHERIC HUMIDITIES. RESULTS EXPRESSED AS AVERAGE WIDTH OF 25 STOMATA CHOSEN AT RANDOM, USING SEVERAL LEAVES

	PERCENTAGE RELATIVE HUMIDITY						
	PELARGONIUM			TOMATO			
	75	50	10	80	50	30	10
Width in mm....	0.04	0.01	Closed	0.03	0.02	Closed	Closed

SECOND METHOD.— It was demonstrated by means of microchemical tests that leaves of the plants studied readily fixed carbon dioxide when in relatively dry air and with the stomata apparently closed. In these experiments the leaves of the plants were depleted of starch in the dark. They were then inclosed in chambers and exposed to arc light. Some of the plants were subjected to a relative humidity of 25 per cent, while others were given a relative humidity of 50 per cent. Microchemical examinations made during the following four hours showed the stomata of leaves at a high humidity to be open, while those of leaves at the low humidity appeared to be closed. At the end of four hours of illumination the chlorophyll was extracted with hot alcohol and the leaves tested for starch with the usual iodine test. Starch was stored in the illuminated leaves regardless of differences in humidity or size of the stomatal apertures (fig. 4).

THIRD METHOD.—The results presented are based on tests made by analyzing the leaf tissue of *Pelargonium* and determining the amount of acid-hydrolyzable materials immediately following a period of darkness during which time the starch was completely digested, as indicated by an iodine test. Plants used in these analyses are referred to as initial plants. The plants were then divided into

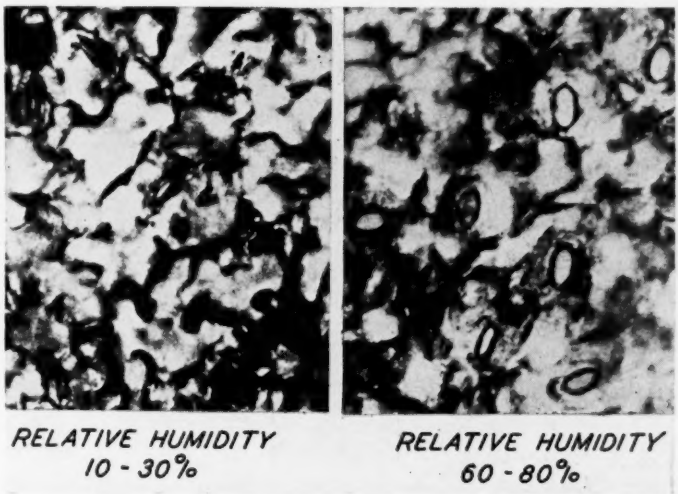

FIG. 3.—Photomicrograph showing size of stomatal apertures in *Pelargonium* leaf exposed to relatively dry air (left), as contrasted with size of stomatal apertures in another leaf of same plant when surrounded by relatively moist air.

two lots and placed under illumination, some of them being exposed to relatively moist and others to relatively dry air. At the end of a six-hour period of illumination, leaves from both lots were again analyzed and the results compared with the initial determinations.

During the interval of illumination the stomata of leaves subjected to a humidity of 10–30 per cent appeared to be closed, but the amount of acid-hydrolyzable materials in these leaves increased approximately 41 per cent over that of the initial plants. Stomata of leaves exposed to a humidity of 60–80 per cent were open and the amount of acid-hydrolyzable materials in these leaves increased

approximately 59 per cent during the period of illumination. It should be stated that results based on the method of carbohydrate analyses used do not, of course, represent all of the compounds of carbon formed in the leaves, and furthermore do not account for carbohydrates which were translocated to other portions of the plants during the period of illumination. It is evident from the results presented, however, that the amount of carbohydrate repre-

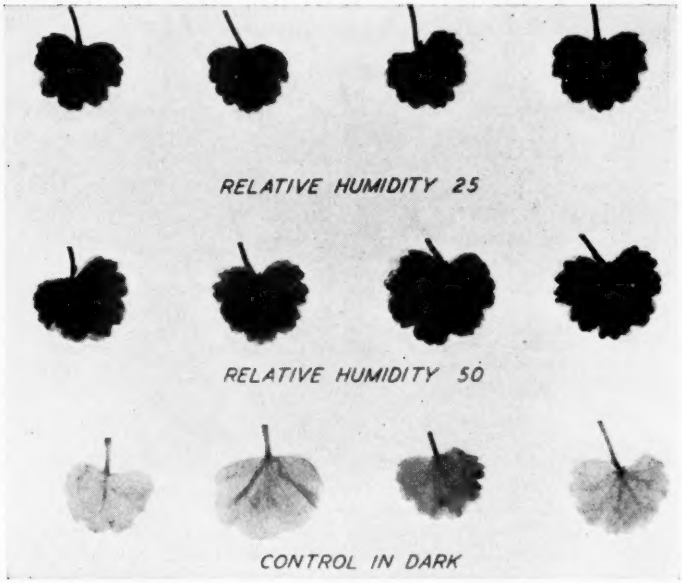

FIG. 4.—Showing starch synthesized by *Pelargonium* leaves at different humidities as compared with other leaves free of starch. The chlorophyll was extracted from the leaves and the starch stained with iodine.

sented in the acid-hydrolyzable fraction, such as sugars, starch, hemicellulose and other polysaccharides, increased in leaves grown in relatively dry air with the stomata apparently closed.

Summary

1. Rates of carbon fixation by leaves of squash, wax bean, cabbage, *Pelargonium*, *Primula*, and tomato plants were not affected either by a rapid decrease in the humidity of the surrounding atmosphere or by a prolonged exposure of 15–20 hours to low humidity.

2. Attached leaves of squash, wax bean, cabbage, *Pelargonium*, *Primula*, and tomato plants remained turgid at moderate temperatures although subjected to an extremely low humidity. The leaves of *Cineraria* plants in some few cases wilted when subjected to dry air, and the loss of turgidity was always accompanied by a decrease in the rate of carbon fixation.

3. Air temperatures above 30° C. retarded the rate of carbon fixation by *Pelargonium* leaves in either moist or dry air.

4. Atmospheric moisture affected the opening of the stomata of tomato and *Pelargonium* leaves. Under the conditions used, the maximum size of stomatal opening generally occurred when the leaves were exposed to relative humidities above 60 per cent. The size of the openings decreased with a decrease in atmospheric humidity and the stomata generally appeared closed in leaves exposed to humidities below 30 per cent.

5. The process of carbon fixation was not greatly affected by wide variations in the size of stomatal openings. The leaves of tomato and *Pelargonium* plants absorbed carbon dioxide and accumulated carbohydrates in appreciable quantities although the stomata appeared to be closed. That these plants absorb carbon dioxide when the stomata appear closed has been demonstrated by means of three methods: (1) the amount of carbon dioxide absorbed by leaves in which the stomata appeared closed was approximately equal to the amount absorbed by the same leaves when the stomata were open; (2) starch was found by means of the usual iodine test to accumulate in leaves exposed to relatively dry air with their stomata apparently closed; (3) acid-hydrolyzable carbohydrates in leaves of *Pelargonium* plants grown in relatively dry air increased approximately 41 per cent during a period of six hours, although the stomata of the leaves appeared to be closed.

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FLORAL DEVELOPMENT AND VASCULAR ANATOMY OF THE FRUIT OF RIBES AUREUM

TSU KIANG YEN

(WITH THIRTY-SEVEN FIGURES)

Introduction

Ribes aureum, the golden flowered currant, is closely related to *R. grossularia*, which GREW (**7**) has figured, showing the ovary as having two placentae, eight vascular bundles arranged as a ring in the fruit wall, and the enlarged cells of the seed coat. MALPIGHI (**10**) indicated that the structure of the gooseberry resembled the pomegranate and that each seed had a similar coating of juice cells. EICHLER (**4**) has given some of the taxonomical characters of *R. aureum*. VELENOVSKY (**15, 16**) mentioned the pericladium of *Ribes*. POHL (**11**) studied the fruit structures of many species of *Ribes*, and special attention was directed to the arillus of *R. aureum*. BUGNON (**2**) observed the trifascicular foliar trace of *R. sanguineum*, which resembles that of *R. aureum*. The present study of *R. aureum* deals with the development of the floral structures as well as with the anatomy of the fruit.

MATERIAL AND METHOD.—Material was collected on the campus of the University of Chicago from the autumn of 1929 until the autumn of 1932, and included series of buds and flowers, together with several stages in fruit development. The usual histological methods and stain technique were followed.

Investigation

DEVELOPMENT OF FLORAL ORGANS

The floral development of a number of plants belonging to the Rosoideae has been studied. GOFF (**5, 6**) studied the cherry, plum, apple, pear, and also called attention to the currant. He found that the flower primordia of the currant were just beginning to appear on July 8 at Madison, Wisconsin.

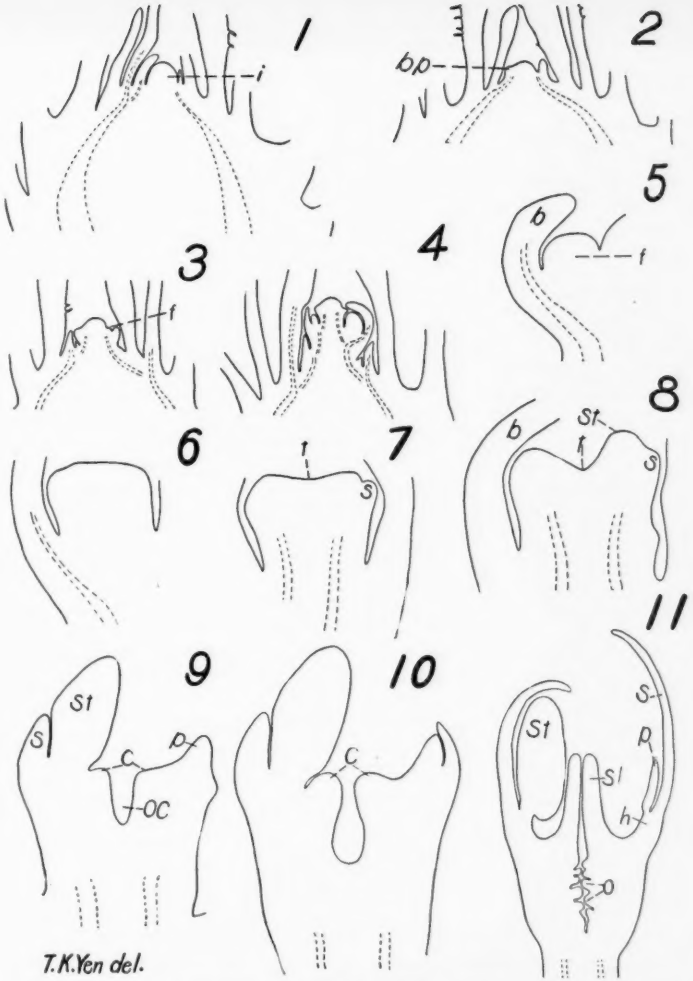
The differentiation of the floral primordia of *R. aureum* is some-

what similar to that of the apple, discussed in detail by BRADFORD (1), KRAUS (9), TUFTS and MORROW (14), and others. The outer bud scales of the dormant bud are five to seven cells in thickness; the epidermis is strongly cutinized, and on the adaxial surface many unicellular hairs are present. Nearly all the epidermal cells, except the hairs, contain tannin. A longitudinal section through a young bud collected August 2, 1931, shows a dome-shaped terminal meristematic portion surrounded by the primordia of spirally arranged bracts or scales. Below this tip the majority of the cells are parenchymatous, although it is possible to discern the structures usually present in a leaf bud (fig. 1).

From the terminal portion of the axis there is developed laterally the more or less ovoid primordium of a bract and soon thereafter a flower primordium arises in its axil. In like manner, bracts with floral primordia in their axils arise acropetally until each inflorescence is composed of six to eleven flower buds, the terminal one always remaining the smallest.

The first appearance of the individual flower is a dome-shaped protuberance (figs. 2–5). The growth of the young primordium is rather more rapid in circumference than in length. After reaching considerable size, elongation of the tip of the axis becomes progressively slower and finally ceases. Meanwhile the tissue near the periphery of the upper portion of the primordium grows more rapidly, and thus the primordium becomes much broadened (fig. 6).

SEPALS.—Five small protuberances are the first structures to arise from the outer circumference of the upper portion of the broadened axis. These are the sepal primordia (fig. 7). They grow into somewhat flattened, elongated structures (fig. 11). Differentiation and development of the epidermal cells are unlike on the two surfaces of the sepal. Those on the abaxial surface grow much larger, are covered by a thick cuticular layer, and some of them mature as well developed guard cells. Those of the adaxial surface have a very thin cuticular layer, are short and hair-like, and are responsible for the velvety appearance of the sepals (3, 13). The mesophyll tissue of the young sepal is composed of three or four layers of cells. They continue to divide until one or two more layers have been added. The cells of a layer which is next to the lower epidermis are rather



FIGS. 1–11.—Fig. 1, longisection of bud collected August 8, 1930; *i*, primordium of inflorescence. Fig. 2, same of bud collected August 8, 1932 showing differentiation of bract; *bp*, primordium of bract. Fig. 3, same of bud collected August 8, 1932 showing differentiation of flower primordium (*f*). Fig. 4, same of inflorescence collected August 22, 1930 showing later stage of fig. 3. Fig. 5, longisection through single flower primordium; *b*, bract; *f*, flower primordium. Fig. 6, same of flower primordium showing broadening. Fig. 7, same of flower primordium showing beginning of cup-like cavity brought about by slower growth of tip of axis than surrounding tissue; *s*, sepal primordium; *t*, tip of axis. Fig. 8, same of later stage than fig. 7 showing differentiation of stamen primordium (*st*). Fig. 9, later stage than fig. 8 showing differentiation of petal primordium (*p*); *c*, carpels; *oc*, ovarian cavity. Fig. 10, later stage than fig. 9 showing centripetal growth of tissue around ovarian cavity. Fig. 11, longisection of young flower bud; *o*, primordia of ovules; *st*, stamen; *sl*, young style; *h*, hypanthium; *p*, petal; *s*, sepal. (Dotted lines show vascular bundles.)

compactly arranged, and plastids may be found in them. The cells of the other layers become parenchymatous, with large intercellular spaces.

STAMENS.—Immediately after the appearance of the primordia of the sepals, a ring of tissue arises just inside them. Soon five protuberances differentiate from the inside of the ring, each being opposite a sepal primordium. They grow upward and soon exceed the sepals in length. These young stamen primordia are ball-like at first (fig. 8) but grow rapidly and elongate (fig. 9). Some of the hypodermal cells divide periclinally and give rise to a single layer of primary sporogenous cells and one layer of primary parietal cells. The wall cells divide several times, resulting in three layers of wall cells and one layer of tapetal cells. The sporogenous cells divide several times and are in the pollen mother cell stage during the winter.

The next spring, microspores are formed. The outermost layer of the wall differentiates as the endothecium. The tapetal cells grow rapidly, soon exceed the wall cells in size, and then begin to degenerate. Before the dehiscence of the anther, they are absorbed completely. The pollen grains are round and smooth, with many germ pores on the surface. Disintegration of the cells between the two loculi begins after the maturation of the pollen grains, and the anther opens as soon as the disintegration of the septum is completed.

PETALS.—The order of development of floral parts is the same as found by SCHERTZ (12) in *Scrophularia*. As soon as the sepals and the stamens have been laid down, the primordia of the petals begin to differentiate (fig. 9). The rudiments of the petals appear similar to those of the stamens and are differentiated from the distal portion of the same ridge of tissue, alternating with the stamens. Three distinct cycles of primordia are now discernible, the stamen cycle on the inside, that of sepals on the outside, and between these two the petal cycle (figs. 9, 10). At this stage the rudiments of the petals are much smaller than those of either the stamens or the sepals. The rounded protuberances soon become ellipsoidal in cross section, and with the exception of the narrower epidermal cells, there are no noted differences between the cells composing them. The basal part of the petal primordia is made up of three or four layers of mesophyll cells, while at its apex only one layer is present. The epidermal cells

of both dorsal and ventral surfaces develop into short, hair-like papillae. At first the petals grow slowly in length (fig. 11), but a short time before the opening of the flower, elongation takes place rapidly and they reach their full length, roofing over the more centrally placed structures. The undiverged basal portions of the sepals, petals, and stamens form a tube, the hypanthium.

CARPELS.—At approximately the time the primordia of stamens and petals begin their development, those of the two carpels are initiated. These appear first as two oppositely placed blunt protuberances just within the cycle of stamens arising from the margin of the cup-shaped receptacle and some distance from the tip of the main floral axis, which is still slowly elongating and enlarging. Soon the whole inner margin of the receptacle grows (figs. 7, 8) and is elevated actively, as is also the tip of the axis except at the central portion and two small areas adjacent to it. The continued elevation of these portions, together with that part of the receptacle lying exterior to them, results in the formation of two communicating carpellary cavities (fig. 9), roofed over by the upper portion of the carpels and surrounded laterally by the ovary walls, the abaxial portions of which, however, are indistinguishable from the tissue of the receptacle from which they remain completely undiverged. The two protuberances first to arise elongate much more rapidly than do the remaining portions of the ovary, and extend upward as development takes place, completely diverged from the remaining portions of the receptacle, sometimes referred to as the calyx tube, and also from each other, except at the base.

WALL OF FRUIT.—A cross section through a very young flower primordium shows practically no differentiation among the cells of the wall of the fruit. All are meristematic. Later procambial strands are readily visible, the vascular bundles appearing closer to the periphery of the receptacle than to the ovarian cavity. The difference between the development of the epidermal cells on the abaxial and adaxial surfaces of the fruit is striking. Those on the outside divide actively. Some of them differentiate as guard cells; others soon become thick walled. A cuticle of considerable thickness is deposited on the surface of the fruit. The epidermal cells on the inside of the ovary wall do not divide so actively, but elongate mainly tangential-

ly. A thin layer of cuticle is observable on them, but later the walls disintegrate and a sheath of gelatinous matter is present in the ripe fruit.

Some of the cells scattered among the parenchymatous tissue of the fruit wall grow more rapidly than others, and chloroplasts develop in them. The walls of these cells become thicker, but after pollination the chloroplasts gradually degenerate, and in a section of the ripe fruit no differences between these cells and the rest can be recognized.

PLACENTA AND OVULES.—As noted previously, there are two carpels, undiverged at their edges up to the time of pollination. These edges develop relatively little toward the center of the axis and thus appear as parietal placentae on opposite sides of the ovarian cavity. During development and maturation of the fruit, they increase in size appreciably. Several rows of ovules arise from them (fig. 11). The inner integument differentiates first but soon is overlapped by the more rapidly growing outer integument. An arillus develops from the funiculus of each ovule. No indication of the arillus can be observed until the diameter of the young fruit exceeds 2 mm. As the fruit grows from 2 to 3 mm. in diameter, the funiculus begins to increase both in number and in size of cells, finally almost enveloping the entire seed. In a section of a ripe fruit, the arillus appears as the pulp of disintegrated parenchymatous cells which fills up most of the space around the seeds. POHL (11) has described the arillus of this fruit.

STIGMA AND STYLE.—How the primordia of the carpels arise has been stated previously. A zone of cells surrounding the stylar canal remain meristematic and serve in part as conductive tissue. The epidermal cells of the most distal, stigmatic surface elongate into papillae. Some of the cells underlying the surface disintegrate a short time before blooming of the flower and form a mucilaginous mass at the surface.

After pollination, the upper portion of the style withers. Following this, two proliferating masses of tissue arise opposite each other on the inner portion of the base of the style. Each is derived from two adjacent carpels. The cells are at first parenchymatous but become suberized during the ripening of the fruit. The proliferations are not

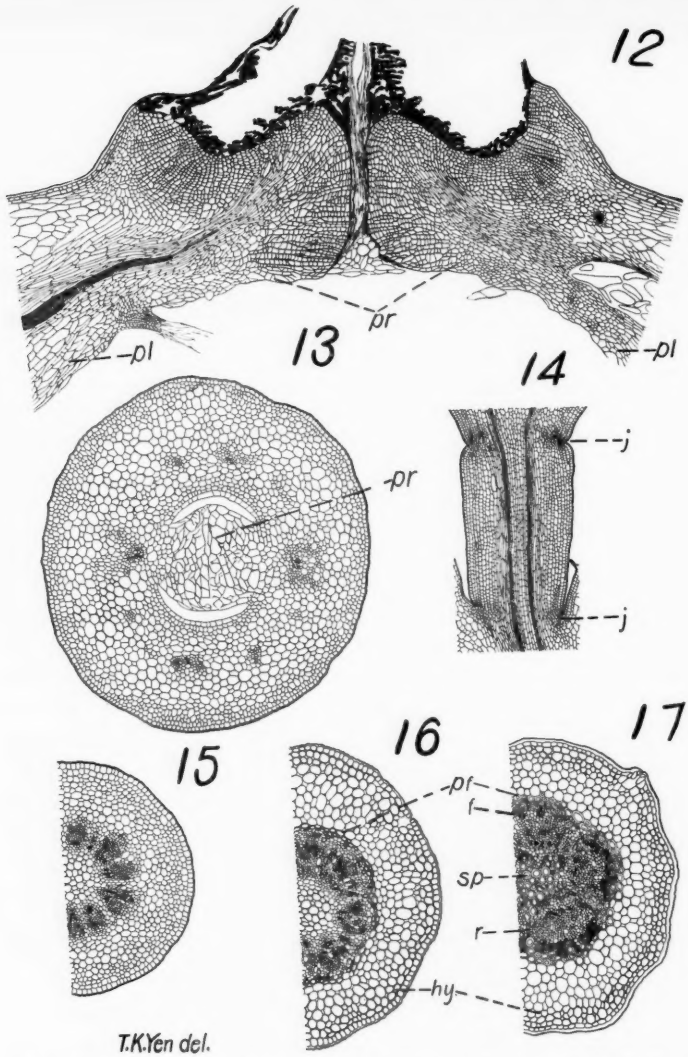
connected with the placentae, although they appear somewhat similar (fig. 13). These two masses of tissue develop rather late. In a very young fruit with a diameter of 3 mm., no trace of the proliferation is observable. When the fruit reaches a diameter of 4 mm., the cells on the inner margin of the base of the style begin to show divisions. The formation of these proliferations is completed in a few days. A cross section through the basal portion of a fruit 5 mm. in diameter shows the stylar cavity almost filled by them (fig. 12).

NECTARY.—The nectary is located in the groove between the hypanthium and the base of the style. In a transverse section of a young flower, it appears first as an active layer of cells next to the epidermis. These cells first divide tangentially but later both tangentially and radially. This activity results in the formation of a ring of tissue consisting of small cells with dense cytoplasm. In a ripe fruit the walls of these cells are suberized.

VASCULAR ANATOMY

BRACT AND PEDICEL.—The vascular bundles in the axis below the base of the inflorescence are arranged in a ring (fig. 18); individual bundles at this point are not clearly distinguishable and are indefinite in number. At a higher level the ring is flattened out into an ellipsoid (fig. 19). Three bundles, one of which is situated at an extremity and the two others located near the middle of the ellipsoid, separate from the ring (fig. 20) and extend into the cortex. They are the main vascular bundles of the bract. This derivation of the three bundles of a bract is the same as in *Ribes sanguineum* as described by BUGNON (2). The lateral veins in the bract of *R. aureum* are more extensive and more profusely branched than is the midrib.

When these three bundles have diverged into the cortex, three gaps are left in the stelar ring (fig. 21), thus separating the ring into two small arcs on either side of the gap and a half circle opposite these two small arcs (figs. 20, 21). The two small arcs of vascular tissue soon diverge into the pedicel of the flower located in the axil of the bract. Above the point of divergence of the bract and the flower, the half ring of the main axis soon becomes a complete one (fig. 26), owing to the differentiation of additional xylem and phloem elements. In like manner this ring of vascular tissue is broken into the



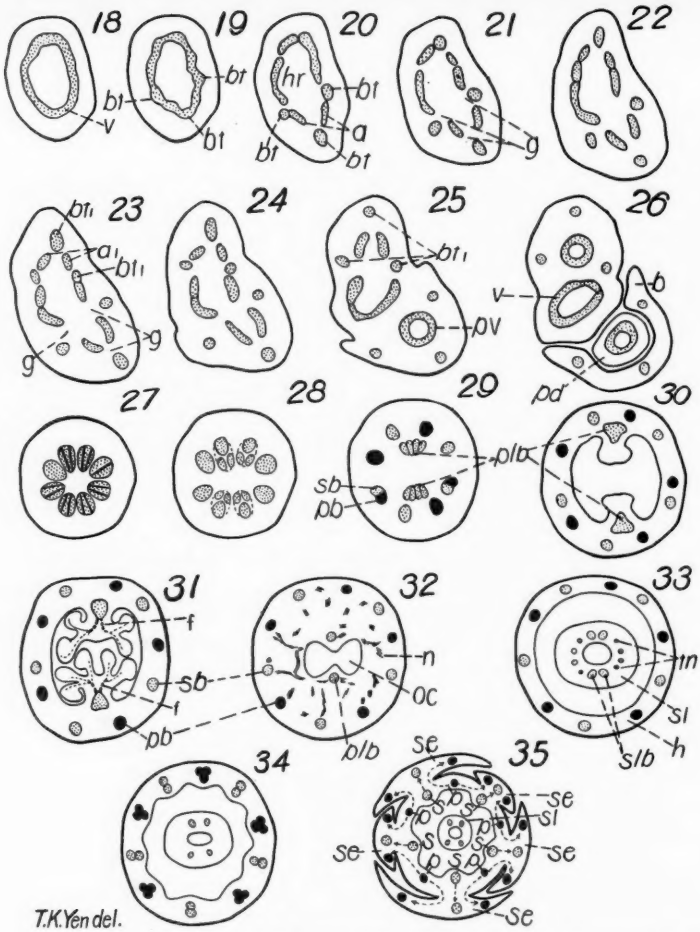
FIGS. 12–17.—Fig. 12, longitudinal view of upper portion of ripe fruit showing proliferations at basal portion of stylar canal; *pl*, placenta; *pr*, proliferations. Fig. 13, cross section of stylar region of young fruit with diameter of 5 mm., showing proliferations (*pr*). Fig. 14, longisection of young pedicel showing differentiation of two joint-like structures (*j*). Fig. 15, cross section of young pedicel showing parenchymatous tissues at that stage. Fig. 16, later stage than fig. 15 showing pericycle and phloem cells beginning to differentiate into fibers and the sclerotic condition of cells of hypodermis (*hy*); *pf*, pericyclic fibers; *f*, phloem fibers. Fig. 17, cross section through pedicel of ripe fruit; *sp*, sclerotic pith; *r*, sclerotic ray cells; *pf*, pericyclic fibers; *f*, phloem fibers.

half ring and two small arcs, which diverge into the flower pedicel, at each successive divergence of a bract and a flower (fig. 26). The differentiation of vascular elements in the pedicel of the flower above the point of divergence of the two small arcs soon closes the two gaps, separating the two arcs, and the ring of vascular tissue is complete.

The stelar ring of the pedicel is composed of eight collateral bundles arranged similarly to those in the stem (figs. 27, 37). Each bundle has from one to three rows of vessels separated by ray cells (fig. 27). Patches of smaller phloem cells separated by larger ray cells are present on the outside of each row of vessels.

BUNDLES OF BASAL PORTION OF FRUIT.—In that portion of the pedicel which flares out at the base of the fruit, the vascular bundles appear somewhat oblique. They are gradually extended toward the periphery of the fruit (figs. 28, 37), parenchymatous cells lying on all sides of them toward the central portion of the fruit as well as toward the outer epidermis. It is not possible to allocate this parenchymatous tissue either to a cortical or pith region since the cells appear so similar in general structure. The bundles are grouped into two crescent-shaped masses, each group composed of four bundles (figs. 28, 37).

PLACENTAL BUNDLES.—Of the four bundles composing the group just mentioned, the two central ones in turn are made up of three smaller bundles (figs. 27, 28). At a slightly higher level the central bundle of each of these groups of three is diverged slightly outward toward the periphery of the fruit, and of these two one is continued upward and finally terminates in a petal, the other in a sepal. The two remaining small bundles of each of these groups of three, making four in all (fig. 28), are diverged toward the carpellary cavity (fig. 29). They approach each other very closely and continue upward as the vascular system in one of the placentae (figs. 29–35). The other four bundles of the two groups lying at the tip of the crescents are not divided into smaller bundles at this level of the fruit (fig. 28), but extend toward the periphery of the fruit wall, so that at this level there are eight bundles in a circle at the periphery, and four placental bundles in two groups near the center (fig. 28). Each placental bundle is diverged into a number of lateral veins from which many vein-



FIGS. 18–35.—Fig. 18, diagram of cross section of base of axis of inflorescence showing vascular cylinder (*v*) as complete ring. Fig. 19, diagram of cross section of axis of inflorescence slightly above that of fig. 18 showing differentiation of bract traces (*bt*). Fig. 20, same, a little above fig. 19, showing vascular cylinder broken into the three traces of bract (*bt*), three gaps, two small arcs (*a*), and a half ring (*hr*). Fig. 21, same slightly above fig. 20, showing the three gaps (*g*). Fig. 22, bract traces extending toward periphery. Fig. 23, differentiation of another bract and floral primordium slightly above fig. 22; *bt*, bract traces of another bract; *al*, small arcs of another primordium. Fig. 24, diagram a little above fig. 23 showing traces extending more to periphery of cortex. Fig. 25, two small arcs of first flower primordium becoming a ring; *pv*, vascular ring of pedicel. Fig. 26, diagram of cross section through node of inflorescence axis showing bract (*b*), pedicel (*pd*), and complete vascular ring of axis (*v*). Fig. 27, cross sectional

lets are derived. These veinlets are the vascular bundles to the ovules (fig. 31). The lateral veins of a main placental bundle are arranged in two vertical rows parallel to each other. The veinlets of the two rows remain distinctly separate and each placenta is therefore recognizable by its venation, as it were, as consisting of two halves (fig. 31).

BUNDLES OF STAMENS, SEPALS, AND PETALS.—At a level slightly higher than that at which the placental bundles become definitely recognizable, two of the peripheral bundles located at the extremities of one of the crescent-shaped groups are divided to form two bundles each (fig. 29). Of the four bundles resulting, two finally terminate in the petals, two in the sepals. Thus ten vascular bundles are formed in a ring near the periphery of the wall of the receptacle (fig. 30). These are the primary bundles of the sepals, stamens, and petals. Each bundle may be branched to form one or more small bundles near the top of the fruit. These small branched bundles extend centripetally almost perpendicularly to the main bundles, diverging freely to form a fine network crowning the upper portion of the ovary (figs. 32, 37). The centripetal differentiation of these veinlets progresses almost to the ovarian cavity and a ring of bundles is completed, with the addition of the placental bundles, around this cavity (fig. 37).

In the upper portion of the hypanthium before the divergence of the sepals, petals, and stamens, the bundles are arranged in two con-

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view of pedicel showing eight vascular bundles arranged in ring, six with three rows of vessels, one with two rows, and one with one row. Fig. 28, diagram of pedicel showing eight bundles arranged in two crescent-shaped groups with two bundles in middle of each group dividing into three bundles each. Fig. 29, diagram showing union of four small bundles into placental bundle (*plb*) and two bundles at either extremity of one of the crescents dividing to form two bundles each. Dotted bundles in outer ring of diagram are stamen bundles (*sb*) and black ones are petal bundles (*pb*). Fig. 30, cross sectional diagram through lower portion of fruit showing two placental bundles (*plb*) and ten primary vascular bundles. Fig. 31, diagram of cross section through middle of fruit showing two parallel rows of vascular bundles (*f*) derived from placental bundles; *sb*, stamen bundles; *pb*, petal bundle. Fig. 32, diagram of cross section through upper portion of fruit showing network of bundles (*n*), ovarian cavity (*oc*), and placental bundle (*plb*). Fig. 33, diagram of cross section through hypanthium (*h*); *sl*, style; *tn*, tips of bundles of network; *slb*, stylar bundles. Fig. 34, cross section through upper portion of hypanthium showing divergence of bundles. Fig. 35, diagram through top of flower showing petals and stamens alternately arranged; *se*, sepals on outside, showing bundle derivation; *s*, stamens; *p*, petals; *sl*, style. Arrows show divergence of sepal bundles.

centric rings (fig. 34), those of the petals and stamens in an inner ring alternating with the sepal bundles. Five of the ten main bundles present here are common bundles of the stamens and sepals (fig. 34). These five usually do not branch until the middle part of the hypanthium is reached, where each branches into two (fig. 36),

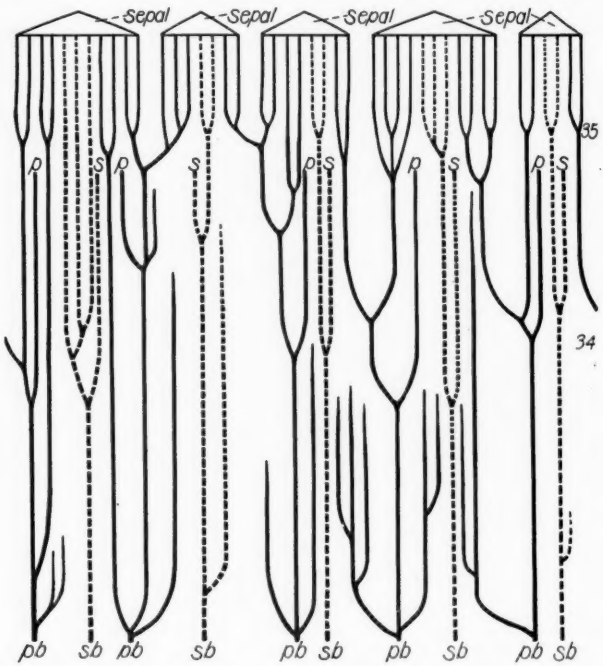

FIG. 36.—Diagram showing venation of petals (*p*), sepals, and stamens (*s*); *pb*, petal bundles; *sb*, stamen bundles.

one extending to the stamen, the other dividing again into two or occasionally more and extending to a sepal (figs. 35, 36). The single bundle which diverges into the stamen remains unbranched.

The other five main bundles terminate in the petals (figs. 34–36). They are arranged alternately between the bundles which are diverged into the stamens, are occasionally rebranched just above the level of the network near the top of the ovary, and these branches

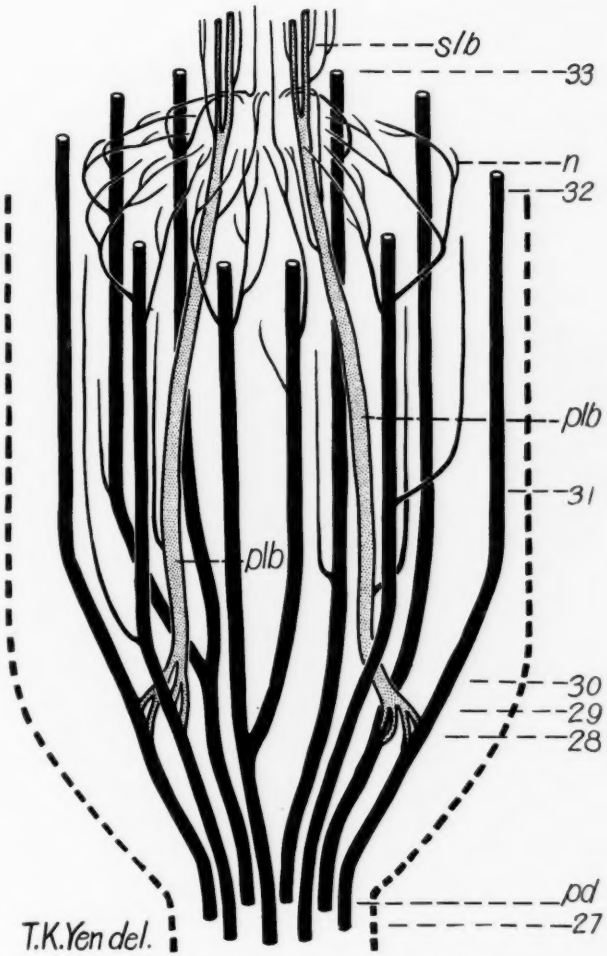

FIG. 37.—Diagram showing venation of a fruit: *slb*, style bundles; *n*, network; *plb*, placental bundles; *pd*, pedicel. Small numbers at right of figs. 36 and 37 indicate places from which the cross section diagrams bearing similar numbers were made.

are diverged into the sepals. Each of these five main petal bundles is divided into two at a point half way up the calyx tube (fig. 36), one becoming the main vascular bundle of the petal. This bundle is rebranched into six or more veins; the other gives rise to two or more branches, each of which is divided into two veinlets which diverge into the lateral portions of either adjacent sepal (figs. 35, 36).

BUNDLES IN STYLE.—Near the base of the style the two placental bundles already described are diverged into two main bundles and several smaller ones. These small bundles are rebranched as a network in the tissue of ovarian walls. They extend through the entire length of the style and are divided into several small veinlets which are traceable almost to the tip of the stigma (fig. 35). Some veinlets other than those derived from the placental bundles are present on either side of the style. These are extensions of the branches from the network near the top of the fruit and are traceable for a short distance in the style above the ovary (fig. 33).

Comparing the floral and vascular anatomy of *Ribes aureum* with the apple (9) in the Rosaceae, the former seemingly represents an advance in the decreased number of stamens and carpels, in the lack of clear demarcation of the ovarian tissue from that of the receptacle, and in the failure of divergence of a distinct vascular supply for the carpels except as represented by the vascular system in the placentae and a portion of the vascular bundles of the styles.

Summary

1. Except for the terminal flower, each flower primordium of *Ribes aureum* arises in the axil of a bract. It is a dome-shaped protuberance at first but soon becomes cup-shaped owing to the lateral expansion of the axis.

2. The order of the floral development is sepals, stamens, petals, and carpels.

3. The carpels are undiverged at the bases and two virtually parietal placentae are present.

4. The inner integument of the ovule is initiated first but is soon overlapped by the outer one. After fertilization, the epidermal cells of the outer integument enlarge and become succulent.

5. The funiculus enlarges as an arillus, the cells of which disinte-

grate into a pulpy mass almost filling the space surrounding the seeds.

6. The two placenta-like proliferations derived from either side of the base of the stylar canal have no connection with the placentae.

7. Two joint-like structures are found at either end of the pedicel.

8. The vascular bundles of the pedicel are eight in number. They divide to form two crescent-shaped groups in the fruit wall, each of four bundles.

9. A placental bundle is derived from the two bundles at the center of each crescent. Each placental bundle gives rise to two vertical rows of veins which are parallel and remain distinctly separate.

10. Each stamen bundle is diverged into two, one of which extends to the sepal and divides again, the other to the stamen and is undivided.

11. Each petal bundle is alternate with a stamen bundle. It is divided into two, one extending into the petal where it is divided again into three bundles with many veinlets; the other is divided into two, one of each of these diverging into the outer portion of either adjacent sepal.

12. The bundles of each sepal are derived from three different origins, those in the center from a bundle common with the stamen bundle for some distance, and those on either side diverged from adjacent bundles terminating in the petals.

13. The bundles of the style are derived primarily from the placental bundles. A few small bundles are diverged upward from the network of bundles at the top of the ovary.

14. The vascular bundles in the fruit wall are arranged in a single ring, which makes impossible a classification of them into carpellary and toral bundles or a segregation of the parenchymatous cells surrounding them into pith and cortex.

National Normal University
Peiping, China

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VASCULAR ANATOMY OF THE SEEDLING OF MELILOTUS ALBA

ELMER B. McMURRY AND EMMA L. FISK

(WITH EIGHTEEN FIGURES)

Introduction

The vascular anatomy of the seedlings in the species of the Leguminosae which have been studied has been found to be exceedingly variable. As white sweet clover has assumed increasing importance economically, it appeared that a study of the transition region in its seedling might prove of interest. The development of the embryo sac and the formation of the embryo in this species was traced by YOUNG (9). COOPER (2) gave an account of macrosporogenesis and early embryology in *Melilotus alba* and *M. officinalis*. Fertilization and the early development of the embryo were traced by COOPER in the variety Redfield Yellow.

Work on the root in the Leguminosae dates back to the classical studies of DE CANDOLLE, VAN TIEGHEM, NÄGELI, and others. According to VAN TIEGHEM (5), NÄGELI in 1858 referred to the centripetal development of the three or four xylem strands in the roots of *Baptisia* and *Lathyrus*. He pointed out that the arrangement of the vascular tissues in the root was the reverse of that of the stem. VAN TIEGHEM (5) stated that the anatomical limit of root and stem is the place where the change from the alternate to the superposed position of the phloem in relation to the xylem occurs, and where the xylem rotates from a centripetal to a centrifugal type.

VAN TIEGHEM (6) described the roots in a number of species in the Leguminosae, including *Medicago sativa*. In regard to the number of xylem strands in the roots in the family, he stated, "Quant au type numérique, il varie dans la famille; fixe quand il est de deux, trois, ou quatre." GERARD (3) referred to the structure of the seedling in *Medicago falcata*, a member of the Trifolieae. VAN TIEGHEM (7) observed that the protoxylem strands of the root migrate to form the many bundles of the stem of *Medicago*.

COMPTON (1) described *Melilotus arvensis* as having a triarch protostele in the root, which changes to diarchy in the hypocotyl by the disappearance of one of the protoxylem ridges. One of the three phloem groups divides to form a fourth phloem strand. He stated that the vascular supply to each cotyledon consists of a "tangential row of bundles, in the middle the vestigial protoxylem, on either side of this a wedge-shaped metaxylem group, and on the extremities of the row the phloems." COMPTON described briefly and figured stages in the structure of the seedling in several other members of the Trifolieae, including *Ononis biflora*, *O. rotundifolia*, *Trifolium arvense*, *T. hybridum*, and several species of *Trigonella* and *Medicago*.

WINTER (8) has described the vascular anatomy of young plants of *Medicago sativa*. She stated, "each cotyledonary trace is made up of a polar xylem strand and a part of each lateral xylem strand" and "cotyledonary traces, as they pass into the cotyledons, are composed of triad bundles, the median portion being a polar protoxylem strand." The change to the collateral endarch bundle occurs in the midrib strand in each cotyledon.

MATERIALS AND METHODS.—Entire plants of *Melilotus alba*, varying from one to sixty days in age, were killed and fixed in Flemming's medium solution, Licent's fixative, Rawlins' fixative, or in chromo-acetic acid. Very satisfactory results were obtained with the use of butyl alcohol as a dehydrating and clearing agent. In the case of younger seedlings, serial sections of entire plants were used.

Observations

SEED AND SEEDLING

The mature seed is characterized by a curved embryo in which the cotyledons and hypocotyl lie parallel with one another (2). The cotyledons are flat and leaflike at maturity and are filled with reserve foods. No endosperm is present in the mature seed.

The seedling is epigeal in development, in that an arched hypocotyl pulls the cotyledons above ground and the latter become photosynthetic. The blades of the cotyledons are oval in shape and the petioles reach a length of 1–2 mm. A unifoliate leaf with stipules develops next above the cotyledons; the third node is characterized

by a typical trifoliate leaf with stipules. A small branch arises early in the axil of the unifoliate leaf, followed by a second in the axil of the first trifoliate leaf. Normally the buds in the axils of the cotyledons do not develop into branches the first year.

Primary root

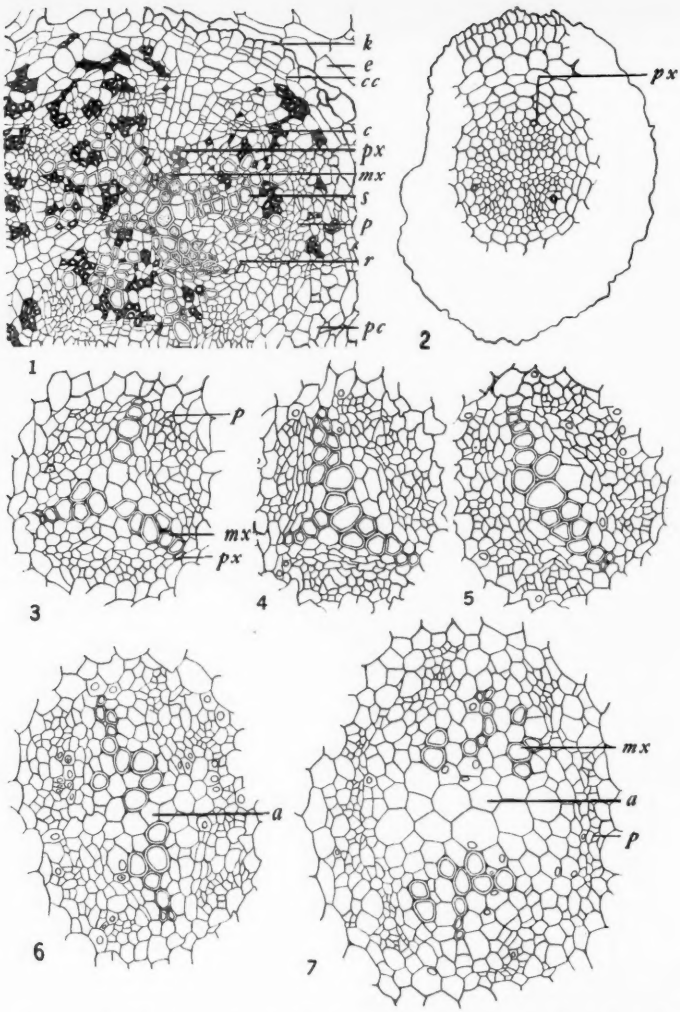
The primary root is characterized by a triarch protostele with three phloem strands alternating with the exarch xylem rays (fig. 3). The outermost cells of the phloem nearest to the protoxylem differentiate as fibers by the time the seedling is seven to nine days old. A pericycle one to two cells in thickness limits the stele; this in turn is surrounded by a row of endodermal cells characterized by Casparian strips. The cortex, which is only several cells in thickness, appears relatively inconspicuous. Secondary roots arise early in the pericycle opposite the protoxylem.

Cambial activity begins in the primary root when the seedling is seven to eight days old, and fibers differentiate early among the conducting elements of the xylem and phloem (fig. 1). This conspicuous development of fibers in the young root is found to be characteristic of a number of species in the Leguminosae. As cambial activity continues, conspicuous parenchymatous rays are differentiated opposite the three primary xylem ridges (fig. 1). A cork cambium arises early in the pericycle and the cortex becomes crushed and soon disappears. The older root is protected by a prominent layer of cork.

Hypocotyl

Serial sections from plants four to seven days old, in which the primary tissues were differentiating and in which there was little or no evidence of cambial activity, were used first in the study of the transition region. This was supplemented by a study of successively older plants up to three or four weeks in age, in which a considerable amount of secondary tissue was evident.

One of the first indications of a change from the typical triarch protostele of the root to the diarch condition in the hypocotyl is noted in the primary xylem elements. In many seedlings approximately four days old, at the base of the hypocotyl one of the xylem strands appears shorter than the other two (fig. 4); at successively



FIGS. 1–7.*—Fig. 1, transverse section of portion of primary root from plant three weeks old, showing primary and secondary tissues; ×170. Figs. 2–7, transverse sections from seedling four days old; fig. 2, primary root showing differentiation of protoxylem, ×170; fig. 3, triarch protostele of root showing differentiation of metaxylem and phloem, ×360; fig. 4, section at base of hypocotyl showing transition from triarchy to diarchy, ×360; fig. 5, diarch protostele at slightly higher level than in fig. 4, ×360; fig. 6, stele in upper hypocotyl showing parenchymatous cells among thick walled elements of xylem, ×360; fig. 7, lateral differentiation of metaxylem, increased number of strands of phloem, and development of pith in upper hypocotyl, ×360.

* All drawings were made with the aid of a projectoscope. The following abbreviations are used on all illustrations: *px*, protoxylem; *mx*, metaxylem; *s*, secondary xylem; *p*, phloem; *pc*, pericycle; *e*, endodermis; *c*, cambium; *cc*, cork cambium; *k*, cork; *r*, ray; *a*, parenchyma; *l*, collenchyma; *ax*, cotyledonary axis; *col*, cotyledon; *ec*, epicotyl; *uni*, unifoliate leaf; *b*, bud; *m*, median trace; *la*, lateral trace.

higher levels fewer elements are differentiated in this strand and it ultimately disappears (fig. 5). This change is accompanied by a gradual orientation (fig. 4) of one of the other two protoxylem points into a position such that a typical diarch protostele results (fig. 5). At the levels where the diarch condition is evident, the stele appears oval in cross section. This diarch arrangement extends through the major portion of the hypocotyl.

In the upper hypocotyl, however, some of the elements of the metaxylem are oriented laterally with parenchymatous cells among them; a few metaxylem elements remain constantly in association with the protoxylem (fig. 6). At successively higher levels there is a gradual change in the position of the "migrating" elements of the metaxylem, until just below the cotyledonary node they appear in a position lateral to and on either side of the protoxylem points (fig. 7). These two primary groups of xylem elements form the traces to the cotyledons. The parenchymatous cells form a conspicuous pith, the stele appears considerably enlarged in transverse section, and the cells of the pericycle have increased in number (fig. 7).

Changes in the primary strands of phloem accompany the changes in the xylem. At the level in the lower hypocotyl at which one of the xylem strands becomes abortive, each group of phloem elements is flattened tangentially (fig. 4) and at higher levels is separated into two or more strands. In young seedlings at the diarch level there are usually evident on either side of the xylem plate three or four groups of small cells, which are apparently undifferentiated elements of the phloem (fig. 5). At higher levels those phloem strands nearest the protoxylem groups in the axial plane become associated with the adjacent xylem in the formation of the cotyledonary traces (fig. 7). Other groups of meristematic tissue in the stele mature later, as parts of the collateral bundles which are continuous with the bundles of the epicotyl.

A second type of reorientation of the xylem different from the preceding was found to occur in other seedlings with about equal frequency. In these, as serial sections at successively higher levels are examined, the earliest indication of transition in the hypocotyl is the differentiation of two of the protoxylem groups in a position to form the cotyledonary axis, and simultaneously several metaxylem ele-

ments differentiate opposite the third protoxylem group. At higher levels these metaxylem elements become more numerous and a tetrarch protostele results (fig. 8), with the rays on the cotyledonary and intercotyledonary axes at right angles to one another. However, spiral and annular elements characteristic of protoxylem are not recognizable in the new, fourth arm. At the levels where a tetrarch condition prevails in some plants, a few parenchymatous cells are found among the thick walled elements.

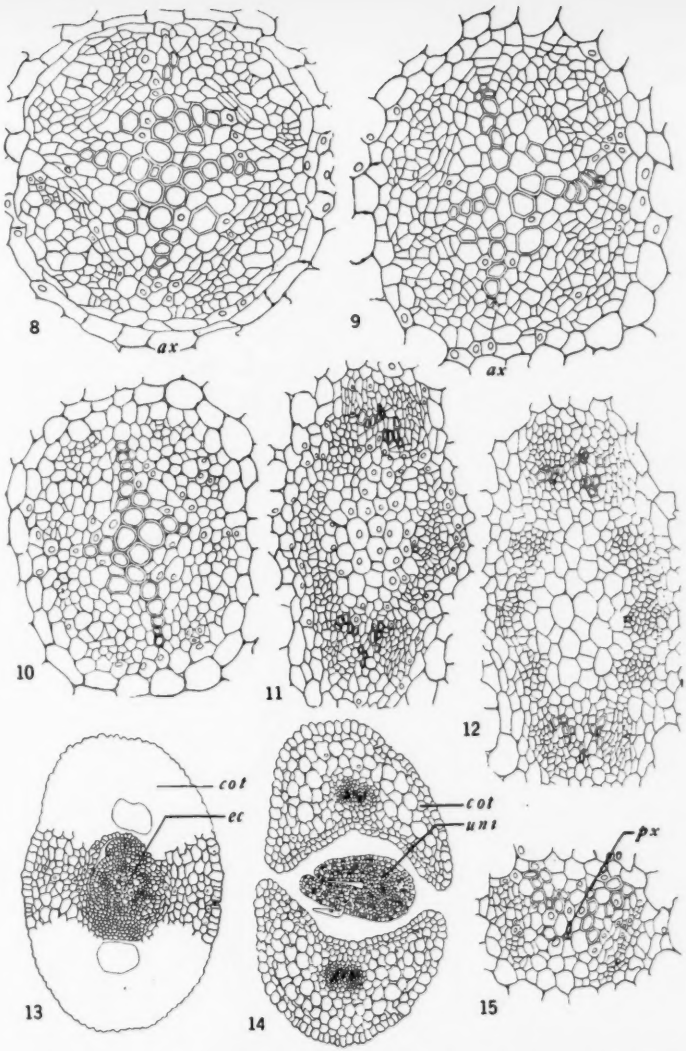
In this second type of seedling tetrarchy gives place rapidly at higher levels to diarchy, as fewer xylem elements differentiate in the last formed ray, accompanied by the lack of differentiation of the original exarch strand opposite (fig. 10). A diarch protostele results, or in some instances a diarch plate with numerous thin walled cells differentiating among the thick walled xylem elements. With the increase in number of cells in the pith at higher levels, the two polar groups with the less closely associated metaxylem elements now separate and become part of the traces to the cotyledons, as in the first type of seedling. The changes in the phloem of the tetrarch type in general appear to be similar to those in the seedlings first described.

Two plants were studied which show an intermediate condition between the two types described, in that only a weak fourth xylem ray differentiates (fig. 9). At a slightly higher level a distinctly diarch condition is noted.

Cotyledons and cotyledonary traces

The protoxylem strands in the axial plane, together with the associated metaxylem and groups of phloem, constitute the traces to the cotyledons. Each trace appears as two collateral strands made up of phloem and metaxylem, with several protoxylem elements between the two groups of metaxylem, thus approaching a mesarch condition (figs. 11, 12). Such a trace was described by Thomas (4) as a "double bundle" and by Compton (1) as a "triad."

As each trace diverges from the stele, a gap in the meristematic cylinder is left (fig. 12). The cotyledons become distinct from the axis as an ensheathing tube. In seedlings several days old, the em-



FIGS. 8–15.—Fig. 8, transverse section of portion of lower hypocotyl from seedling seven days old, showing tetrarchy, ×300; fig. 9, transverse section from hypocotyl of another seedling showing weak development of fourth xylem arm, ×300; fig. 10, section of hypocotyl at level above that of fig. 8, showing reduction of xylem arms in intercotyledonary plane in transition from tetrarchy to diarchy, ×300; fig. 11, siphonostele in hypocotyl just below cotyledonary node in seedling four days old, differentiation of xylem of trace to epicotyl, ×150; fig. 12, divergence of cotyledonary traces from stele, early differentiation of six traces to epicotyl, ×150; fig. 13, differentiation of cotyledons from axis in seedling four days old and origin of cotyledonary buds, ×90; fig. 14, section of seedling four days old showing petioles of cotyledons and blade of unifoliate leaf, ×90; fig. 15, enlarged bundle of cotyledonary petiole, showing collateral arrangement of phloem and metaxylem strands, with protoxylem between the metaxylem groups approaching an endarch condition, ×300.

bryonic cotyledonary buds may be distinguished at this level (fig. 13). The cotyledonary petioles separate from each other on one side at a lower level than on the other.

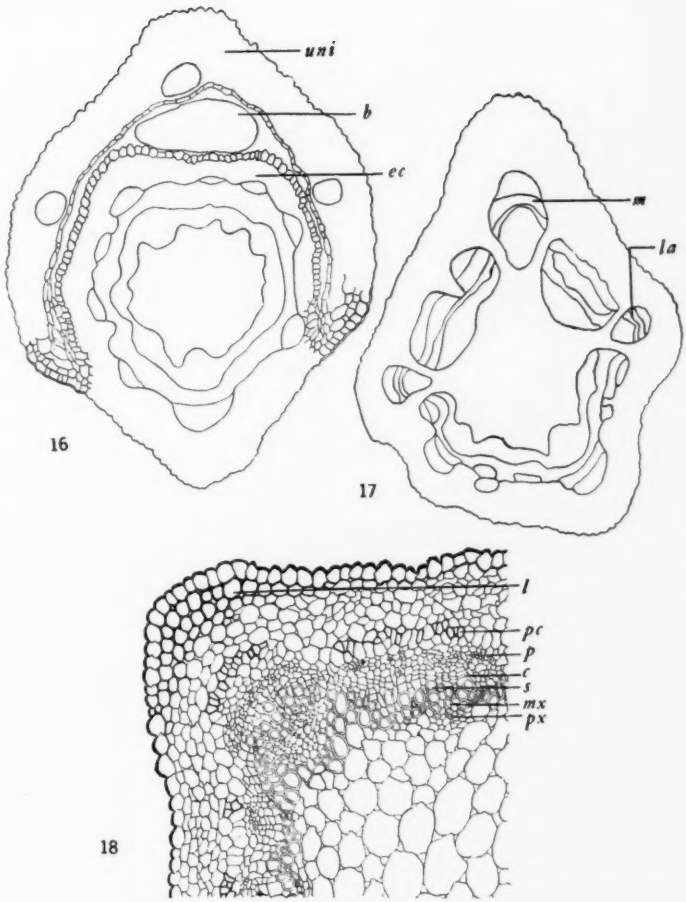
Each trace continues as a single strand through the petiole (figs. 14, 15), and at the base of the blade of the cotyledon the xylem becomes endarch by differentiation of a single rather than of two metaxylem groups and by the further change in position of the protoxylem. A collateral endarch bundle results, in which the protoxylem is directed toward the adaxial surface of the blade. Thus the transition from the exarch to the endarch condition of the xylem is completed in the cotyledons. Branches of the main bundles form numerous vascular strands in the cotyledonary blade.

Epicotyl

In seedlings four to seven days old the epicotyl appears as a mass of meristematic tissue from which the unifoliate leaf is beginning to differentiate. The traces to this leaf appear as small groups of embryonic cells. In seedlings ten to twenty days old a bud may be distinguished in the axil of the unifoliate leaf, and the median and two lateral bundles of the petiole are well differentiated (fig. 16). The stem, which appears circular in cross section in the first internode, becomes square in the third internode, a condition which is typical of the mature stem (figs. 16–18). By the time the seedling is three weeks old a typical dictyostele with endarch collateral bundles is developed in the third internode (fig. 18). In the stem there are prominent fibers in the pericycle over the vascular bundles. The gradual outward differentiation of these fibers may be traced in seedlings ten or more days old from the root through the hypocotyl, where the pericycle becomes more prominent and where many of its cells differentiate as fibers.

Leaf traces

In a cross section of the upper hypocotyl of a seedling four days old there is noted an isolated xylem element which is in no way connected with the two polar xylem rays of the root which supply the cotyledons (figs. 11, 12). This xylem element differentiates directly above the position of the original third protoxylem point or with



FIGS. 16–18.—Fig. 16, diagram of transverse section of second node from plant three weeks old, showing separation of petiole of unifoliate leaf from axis, with median and lateral bundles differentiated and bud in axil of leaf; fig. 17, diagram of transverse section of second internode, showing divergence from stele of median and lateral traces to first trifoliate leaf; fig. 18, transverse section of third internode from plant three weeks old, showing dictyostele, ×340.

equal frequency on the opposite side of the stele. In the young seedling there is no vascular connection between the exarch strands and this developing element; for a short distance above the abortive exarch strand the cells in this region are undifferentiated. In so far as determined, the variation in position of the new xylem element is found in either the type of seedling in which the transition is directly from triarchy to diarchy or the type in which an intermediate tetrarchy occurs. Further study might reveal a relationship between the position of this element and the external anatomy of the seedling.

This xylem is the first indication of an endarch bundle. As the seedling grows, other xylem cells mature adjacent to the first element, phloem matures from the meristematic group of cells adjacent, and these cells together with those differentiating at higher levels form the main endarch collateral bundle which supplies the unifoliate leaf. This trace is therefore continuous from the upper hypocotyl, through the cotyledonary node and through the first internode.

Other bundles appear in the hypocotyl just below the cotyledons by the time the seedlings have reached an age of seven to ten days. They have their origin in the meristematic cylinder of the hypocotyl (fig. 12). The second bundle to differentiate is an endarch collateral trace in the intercotyledonary axis opposite the one just described. This second bundle becomes the median trace to the first trifoliate leaf at the third node. By the time the seedling is two to three weeks old, at least six endarch collateral traces have differentiated in the upper hypocotyl (fig. 12). The two smaller bundles adjacent to the median trace to the third node ultimately differentiate at right angles at the second node as the lateral traces to the petiole of the unifoliate leaf. Thus three traces supply the unifoliate leaf. These continue as three main bundles through the petiole, anastomose at the base of the blade, and then rebranch. The stipular bundles are continuations of the lateral traces.

The two small bundles on either side of the first developed endarch trace ultimately supply the lateral traces to the first trifoliate leaf. As the traces diverge from the main cylinder in the second internode gaps are apparent (fig. 17). Three leaf traces, therefore, are characteristic of this trifoliate leaf. Branches from the lateral traces differentiate in the stipules.

Although the traces to the epicotyl have their origin in the upper hypocotyl, there appears to be no direct continuity of xylem between the primary strands which extend from primary root, through hypocotyl, and into the cotyledons and the xylem of the endarch traces which arise from meristematic tissue in the young seedling. The undifferentiated strands of phloem in the lower hypocotyl of the young seedling seem to be associated in position with these provascular strands which mature into collateral bundles in the upper hypocotyl and in the older seedling. Because of the meristematic nature of these cells, the exact manner of differentiation of phloem between the root and the endarch bundles is difficult to follow; however there seems to be a closer continuity in the differentiation of phloem between root and stem than in the primary xylem. After cambial activity has begun, the secondary tissues of these regions form a more or less continuous system for the movement of water and food materials.

Conclusion

In *Melilotus alba* the transition from the exarch, triarch, radial arrangement of the vascular tissue of the root is completed in the cotyledons with the formation of endarch collateral traces. The major changes in this transition occur in the upper hypocotyl and in the petioles of the cotyledons. There is a continuous system of primary xylem from root into cotyledonary blades, although one of the original xylem ridges disappears in the lower hypocotyl. The protoxylem, and apparently the metaxylem, of these regions have no direct connection with the primary xylem of the endarch bundles which arise from meristematic tissue in the upper hypocotyl, and which differentiate in the epicotyl as traces to the second and third nodes.

In *Medicago sativa*, WINTER (8) found triarchy, and more rarely tetrarchy, to be characteristic of the primary root, while in the lower hypocotyl tetrarchy invariably occurs. COMPTON (1) noted that in *Melilotus arvensis* triarchy gives place to diarchy in the hypocotyl. The condition in *M. alba* is found to be variable, in that, although triarchy is characteristic of the root, either a diarch or a tetrarch arrangement is found in the lower hypocotyl; however, invariably at a higher level a diarch arrangement occurs. WINTER stated that in

alfalfa "the first vessels in the hypocotyl which are continuous with vessels of the plumule" are produced by cambium. In *M. alba* the collateral endarch traces of the hypocotyl, which continue upward to become the traces to the first plumular leaves, arise from meristematic provascular tissue in the upper hypocotyl in seedlings a few days old. However, the lack of direct connection of the xylem of these collateral bundles with the primary xylem rays of the root and lower hypocotyl in the young seedling resembles the condition found in alfalfa.

The account given by COMPTON (1) of the seedling anatomy of certain species of *Ononis*, *Trifolium*, *Trigonella*, and *Medicago* would indicate that, although variations do occur, there are striking similarities between the mode of transition in these plants and that found in the present investigation on *Melilotus alba*. These similarities, together with the condition described by WINTER in *Medicago sativa*, are indicative of an anatomical relationship between these species of the Trifolieae, and serve as further evidence for a close phylogenetic relationship between the members of this tribe of the Leguminosae.

Summary

1. The primary root of *Melilotus alba* is characterized by a triarch radial protostele.

2. The first indication of a change from the triarch exarch arrangement of the xylem of the primary root in the young seedling is the development of diarchy or tetrarchy in the lower hypocotyl.

3. If tetrarchy occurs, it is invariably replaced at a higher level in the hypocotyl by diarchy.

4. The exarch ridge of xylem on the intercotyledonary axis gradually disappears at higher levels in the hypocotyl, and has no direct connection with the primary xylem of cotyledonary or plumular traces.

5. The exarch-endarch transition is continued in the formation of the cotyledonary traces and is completed in the main vascular bundles at the base of the cotyledonary blades.

6. A conspicuous pith differentiates in the hypocotyl, the stele enlarges, the cortex becomes more prominent, and a cutinized epider-

mis is present in this region in the young seedling. An endodermis with Casparian strips is present throughout the greater length of the hypocotyl.

7. A continuous, primary vascular system exists from root, through hypocotyl, into cotyledons.

8. The median vascular bundle which supplies the unifoliate leaf at the second node develops from meristematic tissue in a seedling several days old. The first indication of the differentiation of this collateral endarch bundle is the appearance of a single xylem element in the upper hypocotyl on the intercotyledonary axis, above the disappearing protoxylem of the third ray or on the opposite side.

9. In seedlings seven to ten days old, the median trace to the first trifoliate leaf at the third node, as well as the four bundles which continue upward to form the two lateral traces to the unifoliate leaf and the first trifoliate leaf, respectively, are well differentiated as endarch collateral bundles in the upper hypocotyl. These six primary bundles arise from meristematic tissue and appear to have no direct connection with the primary exarch strands in the lower hypocotyl.

10. Buds arise early in the axils of the cotyledons, the unifoliate leaf, and the first trifoliate leaf. The cotyledonary buds do not ordinarily develop into branches the first season.

11. Cambial activity occurs early in the primary root; fibers are prominent in the secondary xylem and phloem. As a result of cork cambium, which arises in the pericycle, a prominent cork is formed and the cortex of the root disappears.

12. The square stem is characterized by a dictyostele, with collateral endarch bundles.

13. The similarity between the method of transition from root to stem structure in *Melilotus alba* and the types described in *M. arvensis*, *Medicago*, *Trigonella*, *Ononis*, and *Trifolium* indicates a close structural and phylogenetic relationship between these species in the Trifolieae.

University of Wisconsin
Madison, Wisconsin

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EMBRYOGENY OF SPECIES OF PODOCARPUS OF THE SUBGENUS STACHYCARPUS

J. T. BUCHHOLZ

(WITH TWENTY-ONE FIGURES)

Introduction

The genus *Podocarpus* has been divided into the two subgenera, *Stachycarpus* and *Protopodocarpus*. In 1847, ENDLICHER described the subgenus *Stachycarpus*. The writer has made a study of the embryogeny of three species of the latter subgenus, *Podocarpus spicatus* R. Br., *P. ferrugineus* Don., and *P. usambarensis* Pilger.

The material for this study was obtained from three sources. *Podocarpus spicatus* was collected in New Zealand in 1911 by Dr. E. W. SINNOTT; *P. ferrugineus* was supplied by Mr. H. W. LAWTON, of Wellington, New Zealand; and *Podocarpus usambarensis* Pilger was obtained from Jamaica, where it is grown as an introduced species. While this latter material does not agree in every detail with PILGER'S (**6**) description of *P. usambarensis*, the large seeds and the leaves exclude it from the only other species that it could be, *P. falcatus*.

SINNOTT (**8**), in his description and figures of the proembryo of *Podocarpus spicatus*, includes the first sporophytic mitosis, the descent of the four free nuclei to the lower end of the archegonium, the sixteen free nuclei at the pointed end of the archegonium before wall formation, and a proembryo soon after wall formation in which the prosuspensor cells are just beginning to elongate. For *P. ferrugineus*, SINNOTT shows four free nuclei of the proembryo in the upper central part of the archegonium, a stage after their migration to the pointed end of the egg, and a section showing the embryonic tips of two neighboring embryo systems after a period of suspensor elongation. The writer (**2**) found several additional stages in the early development of the embryo of *P. spicatus*, but the later embryogeny, including the stages of tissue organization and development of cotyledons, remains to be worked out.

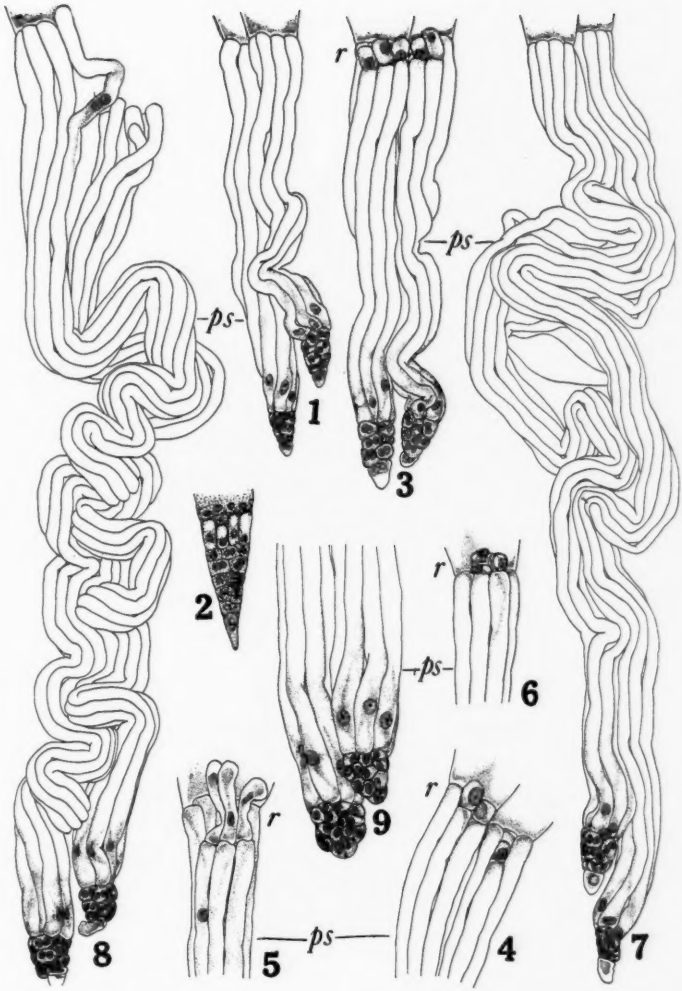
Investigation

Figure 1 shows two adjacent embryo systems of *Podocarpus spicatus* at an early stage, in which the prosuspensors have slightly elongated. This pair of zygotes shows no rosette cells. There are seven to nine prosuspensor cells, each of which has a nucleus in the lower end of the elongated cell. Each embryo system bears a group of nine to twelve binucleate embryonic cells below the prosuspensors. The writer has stated previously (2) that this binucleate condition of the early embryonic cells seems to be characteristic of the subfamilies Podocarpoideae and Phyllocladoideae. This condition has been found in twelve species. A detailed study of the embryogeny of *Dacrydium cupressinum* (4) has more recently been made.

Figure 2 shows a dissected proembryo of *Podocarpus spicatus*. The structure of the proembryo of *P. ferrugineus*, reported by SINNOTT, was similar to that of *P. spicatus*. In both of these species the proembryo is confined to the lower one-fourth of the slender long-pointed archegonium.

The pair of embryo systems in figure 3 shows several rosette cells and the terminal cap cells which are beginning to disintegrate. Most of the embryo systems of similar age, and nearly all of the older embryos, show no rosette cells (figs. 7, 8). It is possible that this is because the rosette cells collapse and disappear at an early stage of embryo development. The rosette groups of cells shown in figures 4–6 are from zygotes of about the same stage as the zygotes in figures 1 and 3. Figures 5 and 6 show some of the rosette cells beginning to disorganize. The rosette cells are uninucleate, and it is probable that they may be prosuspensor cells which failed to elongate. It is believed that the free nuclei above the prosuspensor in the proembryo of figure 2 soon disorganize and disappear, since they were not found in older material.

The binucleate terminal embryo initials which form the cap cells become aborted and are crushed as the prosuspensor pushes the terminal group of cells downward into the gametophyte. As the prosuspensor elongates, several of the terminal embryo cells may be successively crushed (fig. 8). During the growth of the proembryo the prosuspensor becomes twisted and coiled, and very long. Not all of the elongating prosuspensor cells become the same length. Fewer



FIGS. 1–9.—*Podocarpus spicatus:* figs 3–6, variations in rosette cells; figs. 1, 7, 8, absence of rosette cells (*ps*, prosuspensor; *r*, rosette cells). ×100.

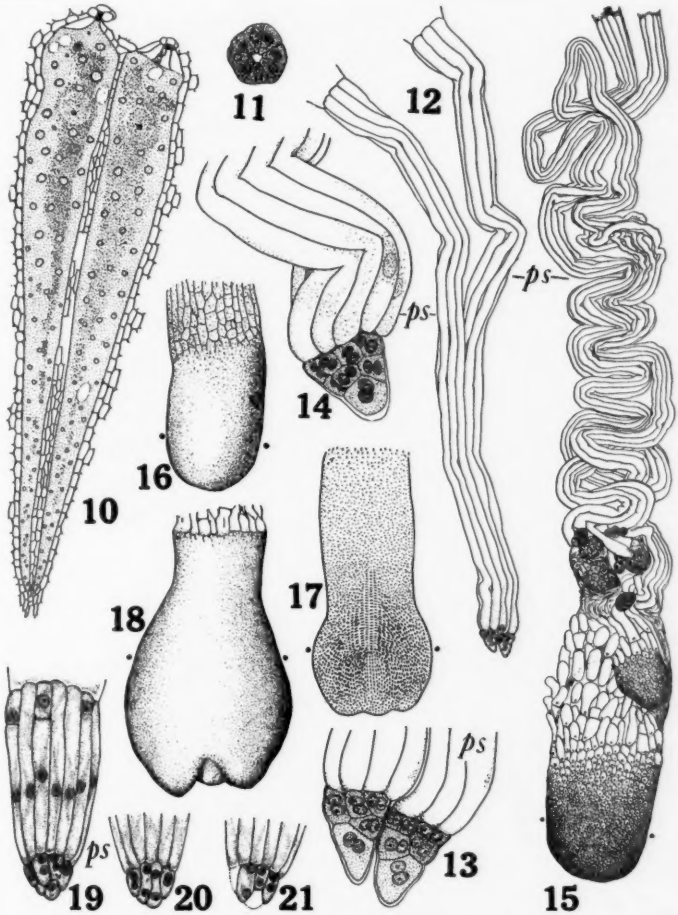
than six or seven cells of an elongated prosuspensor, consisting near the distal end of nine or more cells and lying at the base of the egg, may be joined to the embryonic cells at their proximal end.

As indicated by figures 7 and 8, the embryonic cells remain binucleate for a considerable period during the elongation of the prosuspensor. Ultimately cell plates appear between the two nuclei of each embryo initial or unit, followed by other cell divisions, resulting in a single embryo for each system (fig. 9). Observations have not been made on later stages in the development of the embryo, but it is assumed that the units of each zygote combine to form a single embryo, and that there is no fixed number of these units. One or several of the embryo initials may be broken from the tip of the prosuspensor, and thus an embryo may be formed from only five or six of these initials. On the other hand, there may be as many as ten or more units which form the cylindrical body of the later embryo.

Both *P. spicatus* and *P. ferrugineus* have long-pointed archegonia. Figure 10, from a dissection, shows two adjacent archegonia of the latter species at the time of fertilization. There are many protein granules and vacuoles scattered throughout the cytoplasm of the egg and it is difficult to identify the nuclei. The two archegonia of figure 10 are slightly flattened against each other, so that they appear somewhat narrower in the figure than when viewed from a plane at right angles.

Figure 12 shows the prosuspensors of two neighboring systems of *P. ferrugineus* after they have elongated. Figure 13 is a view of the lower part of figure 12. There are six or seven binucleate units in the embryo at the left, and nine in the one at the right. Figure 14 belongs to another embryo system in which there are ten binucleate cells and the prosuspensor has become more elongated and excessively twisted. These figures show that *P. ferrugineus* has binucleate embryo initials, but unlike *P. spicatus*, the terminal cell in the few specimens examined appears to persist and may contribute to the embryo. Figure 15 shows a considerably older stage of development than figure 14. This material was obtained from a dry seed in which there were more embryos than the number usually found in two zygotes, so in this instance there must have been a cleavage in the group of binucleate units in one of the two zygotes whose prosus-

pensors are still visible above. Evidently here cleavage polyembryony is the exception and simple polyembryogeny is the usual condition.



FIGS. 10–21.—Figs. 10–18, *Podocarpus ferrugineus;* figs. 19–21, *P. usambarensis.* Fig. 11, polar view of neck cells of an archegonium. Figs. 10, 12, ×55; figs. 11, 13, 14, ×210; figs. 15–18, ×40; figs. 19–21, ×100.

From some dry seeds of *P. ferrugineus* there was dissected a suspensor system over 8 mm. in length. The embryo, although broken

off during dissection, was too small to be identified. The seeds of this species seem to require a long rest period before germinating, possibly because the embryo is very small and must undergo considerable development before the seed is ready for germination. Mr. LAWTON, the collector of the seeds, stated that they may require months to germinate. The cause of the delayed germination, however, should be more thoroughly investigated in the region where they grow. A study should be made of the stages of development of the embryo both before and after the seeds are shed. Only a few of the embryos in the several dozen seeds that were dissected showed cotyledons beginning to develop, and in only one of these were the cotyledons longer than those seen in figure 18. The embryo of *P. ferrugineus* in figure 18 is, with two exceptions, in the oldest stage of development of any that could be found in the dry seeds. All of these embryos are immature when compared with the embryos found in ripe seeds of most conifers.

In figure 17 the plerome of the root tip is seen near the center of the spherical part of the embryonic tissue. The shading here approximately indicates the orientation of the cells. The tissue on the side toward the secondary suspensor is the periblem, root cap, and suspensor combined. The upper cells of this cylindrical mass of embryo cells elongate successively to add to the massive secondary suspensor. The cells nearest the tip and at the sides of the plerome constitute the periblem. The root cap region is composed of an undifferentiated mass of periblem and calyptrogen cells, similar to those found in *Cedrus*, which BUCHHOLZ and OLD (5) have called calyptroperiblem.

The plerome of the root tip may be recognized even in the optical sections of cleared whole mounts. In the terminal embryo of figure 15 the tip of the pleromic arc of the root was seen at the level between the two black dots placed at each side of this figure. Black dots also mark the position of this structure in the embryos of figures 16–18. Thus only a small part of the cylindrical embryonic cell mass forms the embryo. It is probable that the entire later embryo is derived from a few, or only one, of the earlier binucleate units, the others giving rise to cells which ultimately contribute to the suspensor and calyptroperiblem.

The primordia of the stem tip and cotyledons are the next structures to develop. The meristem of the stem tip is visible between the two cotyledons in figure 18.

From a study of the embryos of *P. ferrugineus*, dissected from soaked seeds, it appears that there is usually no cleavage polyembryony and therefore no primary suspensor is formed. There is no single-celled structure such as the primary suspensors which may be seen in *Sciadopitys* (3) or *Biota* (2). The prosuspensor elongates greatly, as shown in figure 15. On the end of this, the binucleate group of cells, such as those shown in figures 13 and 14, usually later combines to form a cylindrical mass composed of several hundred cells. When the prosuspensor has reached its maximum length, a massive rope of many embryonal tubes elongates to form the secondary suspensor.

It is possible that the development of the embryo from the binucleate initial cells will be the same in *P. spicatus* (fig. 9) as in *P. ferrugineus*. The embryo system in *P. spicatus* probably does not normally undergo cleavage, and there is no primary suspensor.

Only one early stage in the embryogeny of *P. usambarensis* was found, but this is a very important one, since it shows the organization of the late proembryo (figs. 19–21). Figures 20 and 21 were drawn from the same embryo in successively deeper planes of focus; a total of nine binucleate embryo initials were seen. The embryo initials have become unequal in size, and all of them are oriented in a single tier with their long axes parallel to the axis of the prosuspensor. The cells of the prosuspensor are eighteen to twenty in number and their single nuclei are almost centrally located. Several rosette cells were found above the prosuspensor cells, and as usual there were many free nuclei above the rosette cells which were lost in dissection.

As shown in figures 19–21, the prosuspensor is very massive, consisting at first of twenty or more cells. Dissection of the later stages shows that the embryo of *P. usambarensis* develops a long prosuspensor, which is spirally and irregularly coiled in the cavity between the small terminal embryo and the micropyle. No evidence of cleavage polyembryony was observed in about a dozen dissections of the specimens which were examined. The prosuspensors were greatly

elongated. In the later embryo a distinct stem tip meristem, similar to that of *P. ferrugineus*, was recognizable, but the embryos of mature seeds were well developed.

There is no apical initial cell in the embryo of *P. ferrugineus* or *P. spicatus*, and furthermore there seems to be no apical cell growth in the embryonic cell masses at any stage of their development.

PILGER (7) noted the length of the suspensor in *P. amarus*, a species closely related to *P. usambarensis*, which he dissected from a mature seed. This suspensor system, which was 30 mm. long, was spirally coiled in a cavity beginning at the micropyle and was terminated by an immature embryo in which the cotyledons had not begun to develop.

Discussion

The material for comparison of embryo development was not abundant, but the writer was fortunate in having stages of the three species which corresponded closely enough to permit critical comparisons, and to make what seems to be a reasonably accurate reconstruction of the organization of the late proembryo. The range in diversity of formation and development of the embryo, within the restricted group studied, is pronounced, and calls for an explanation of the causes of the diversity. The differences in embryogeny are greater than those found among the genera of other families, or even between other families of the conifers.

A study of the morphology and taxonomy of the three species of *Stachycarpus* seems to indicate that the species representing the two extremes of structural features are *P. spicatus* and *P. usambarensis;* the intermediate form is *P. ferrugineus*.

This subgenus apparently presents a complete series of evolutionary steps in its external morphology. The features of the embryogeny which are known sufficiently to permit comparison show corresponding differences, but there is a question as to whether the internal changes were at all synchronized with the changes in external morphology. Apparently a massive prosuspensor (which develops into a structure of great length, having many twists and coils) and the organization of eight to twelve binucleate embryo units are features common to all three genera, and may therefore represent the

most conservative feature. *P. spicatus* has the binucleate embryo units organized into four, five, or more tiers in the early embryo; *P. ferrugineus*, into three or four; and *P. usambarensis* has them in a single tier. Thus these details in the arrangement of the binucleate units of the embryo initials are more variable features which are of lesser importance phyletically, but significant ontogenetically. *P. usambarensis* has a much larger number of prosuspensor cells than *P. spicatus* and *P. ferrugineus*, while the deciduous cap cells found in the embryos of *P. spicatus* are not present in the other two species.

Apparently an evolutionary series is suggested by these differences in arrangement of binucleate embryo units. Either the many tiers represent an advanced condition and the single tier a primitive condition, or the reverse.

These alternatives must be kept in mind as the embryogeny of other podocarps is studied, for in *Dacrydium* there are only two tiers of binucleate cells, or at most three, and in *P. totara* (**8**) there is only a single tier, represented by a single terminal binucleate cell.

There is also the possibility that these modifications in the organization of the embryo are occasioned by the pronounced differences in the shapes of the archegonia. The archegonia of *P. usambarensis* are numerous, at least four or five in number, and not long pointed; those of *P. spicatus* and *P. ferrugineus* are usually two in number, and extremely narrow and long pointed.

If it is assumed that the changes in internal embryogeny kept pace with evolutionary changes in the external morphology, then it may be expected that the type represented by the large seeded *P. usambarensis* is the most advanced member of the series. Returning to a consideration of the external morphology, it is evident that *P. spicatus* and *P. andinus* (**6**) have a definite ovulate cone axis bearing six to nine or more bracted ovules in spiral succession, the entire spicate arrangement constituting a loose ovulate cone. In *P. ferrugineus* there is usually only a single ovule, which matures on the end of a stalk representing the cone axis.

In both these cases, as SINNOTT has shown, the epimatium, or the morphological equivalent of the cone scale, is fused with and surrounds the ovule so that the ovules appear to have an extra outer integument. These members are borne in the axis of a bract with

vascular connections comparable with those of the bract and scale of the Pinaceae. In *P. usambarensis* as in *P. ferrugineus*, the number of ovules has been reduced to one, which in *P. usambarensis* becomes very large and is borne on the end of a cone axis having many nodes. Thus *P. spicatus* is the least specialized and *P. usambarensis*, with its very large seed, is the most specialized in external morphology—obviously an evolutionary series in which a single stalked ovule has been evolved from a loose spicate strobilus. If the changes in internal embryogeny have kept pace with, and run parallel to, the evolution in the external anatomy, then the embryo of *P. spicatus* (and the unknown embryogeny of *P. andinus*) should present the most primitive condition. On the other hand, we should also consider the cause for the changes in the shape of the archegonia. These changes doubtless affected the organization of the embryo. In their more primitive condition the archegonia were probably not narrow and long pointed, but of the oval type usually found in conifers, a condition which exists in the archegonial group of *P. usambarensis*. We may therefore consider that the pronounced differences in the organization of the late proembryos of these three species have in some way been connected with the change in shape of the archegonia, so that the binucleate embryonic units which are found in a single tier of *P. usambarensis* have become arranged in three or more tiers in the narrowed archegonium of the other species. Thus one may see the possibility of a causal relationship in the modification of the embryogeny from the original type. The embryos of *P. spicatus* and *P. ferrugineus* may therefore represent the specialized condition, and *P. usambarensis* the more primitive condition. These suggestions are provisional, and definite conclusions cannot be reached until more facts concerning the embryogeny of these and other podocarps are available.

The embryo of *P. spicatus* has been compared with that of *Cephalotaxus* by the writer (1) and others in regard to the existence of a deciduous cap cell at the distal end of the embryo. The writer later pointed out that these cap cells in *Podocarpus* are always binucleate, while those of *Cephalotaxus*, as well as its functional embryonic cells, are uninucleate structures. The apparent similarity is

therefore only an interesting parallelism in evolution. Similar cap cells occur also in the embryos of *Sciadopitys* (3). Likewise all the rosette cells of these podocarps abort and disappear early, while those of *Cephalotaxus* usually give rise to rosette embryos.

Another difference between the embryo of *Cephalotaxus* and these embryos of podocarps is in the time of appearance of the stem tip of the latter embryo. The meristem of the stem tip is delayed in *Cephalotaxus* and is not present as an emergent structure in an embryo at the stage found in the mature seed. In *P. ferrugineus* the stem tip is present in the embryo and is recognizable at least as early as the cotyledons. We may expect the embryo of *P. spicatus* to agree with this. Similarly, in the embryo of *P. usambarensis* the meristematic dome of the stem tip is recognizable long before the embryo completes its development within the seed. Thus when the early and late embryogeny of *Cephalotaxus* and these podocarps are compared, little similarity is found between them.

Summary

1. Pronounced differences are found in the appearance and organization of the early embryos of *Podocarpus spicatus*, *P. ferrugineus*, and *P. usambarensis*, which belong to the subgenus *Stachycarpus*.

2. All three species have seven to twelve binucleate embryonic cell units. These units give rise to groups of cells which usually combined to form a single embryo; thus simple polyembryony prevails within this group.

3. The embryogeny of these three species suggests a sequence of evolutionary steps which appears to be opposite to the sequence suggested by study of the external morphology of the seed cone—the reduction series from a loose strobilus to a stalked ovule. Causal morphology may explain the modifications in the development of the embryo as due to changes in the shapes of the archegonia.

4. No apical initial cell could be observed at any stage in the development of the embryo of any of the species.

5. The resemblances between the embryos of *Cephalotaxus* and those of certain podocarps are superficial and do not suggest close phylogenetic connections.

6. In *P. ferrugineus* and *P. usambarensis* the stem tip meristems are organized and recognizable between the cotyledons of the embryos before the seed is mature.

UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

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SECONDARY ROOT HAIRS

M. ELIZABETH PINKERTON

(WITH EIGHT FIGURES)

In certain species of *Tradescantia* heavy-walled root hairs which form a persistent white or brown tomentum upon dried specimens have been noted. It was the purpose of this research to investigate the anatomical features of the roots of the Commelinaceae in particular, as regards the root hairs, and to try to determine the function of the latter.

Literature

As early as 1877 DE BARY (2) distinguished two types of root hairs, transitory "Safthaaren" with contents, and persistent dead, air-filled "Deckenhaaren." SCHWARZ (13) thought that their delicate nature facilitated absorption. The hairs soon became brown, disorganized completely, and at the same time the epidermis died. Their death, however, occurred before periderm formation, was unrelated to it, and was inevitable whether the root was in air, soil, or water. Later he recorded observations of turgid hairs in maize at a distance of 20 cm. from the tip, in apparent contradiction to his previous observations. GRAVIS (4) noted that in *Tradescantia virginica* the "assise pilifere" became suberized early and persisted at the surface of the older roots as empty cells with brown walls and of rather withered structure. It was assumed by KROEMER (8) that there was a secondary state of the "Aufzellen" achieved with the cutinization of cell walls and loss of protoplasm. The occurrence of hairs with thickened walls in a number of widely separated families and their special abundance in xerophytes was mentioned by HESSE (6). He assumed that absorption through them would be impossible owing to the heavy walls, but that an anchorage function was likely.

LEAVITT (9) came to the general conclusion that root hairs had two types of origin. In the simple type any cell of the epidermis might become piliferous, with no apparent predetermination; in the other there was differentiation under the root cap into small wedge-

shaped densely staining cells, which he called "trichoblasts," and larger, lightly staining cells. The former elongated little but usually became papillate, whereas the intervening cells did increase in length but not laterally. Owing to the horizontal extension from the wedge-shaped bases of the trichoblastic cells, the atrichoblastic epidermal cells might be sloughed off. In some plants having the simple origin of epidermal hairs he observed that there was a differentiation of the exodermis into "regular" and "transfusion" cells. The latter, swelling tangentially, forced aside the epidermis and became functionally absorptive. Chemical tests indicated that these transfusion cells had cellulose walls whereas the other hypodermal cells were cutinized. Roots having the trichoblastic mechanism lacked the transfusion apparatus, and phylogenetically the two conditions were generally segregated. According to LEAVITT, the dicotyledons and some monocotyledons (including the Liliiflorae, Spadiflorae, Orchidaceae) have simple epidermal hairs replaced by a transfusion-cell piliferous layer from the cortex; whereas the majority of the monocotyledons have the predetermined trichoblastic layer which is morphologically a cortical member.

HABERLANDT (5) quoted KROEMER to the effect that sometimes the walls of persistent hairs undergo lignification or become impregnated with protective substances of unknown composition. He mentioned specifically long-lived hairs on two epiphytic ferns, *Drymoglossum nummularifolium* and *D. piloselloides*. WATSON (15) merely described the occurrence of root hairs on old roots of *Helianthus rigidus*.

The first attempt to explain the persistence of root hairs upon an anatomical basis was made by JEFFREY and TORREY (7). They maintained that there was a correlation between the presence of persistent hairs and the absence of secondary growth of the root in *Aster*, and that this condition led to a higher absorptive efficiency. MCDOUGALL (10) investigated the prevalence of thick-walled root hairs in the Caesalpiniodae, and stated that the roots appeared like ectotrophic mycorrhiza, and that the individual root hairs were dark brown and did not shrivel. In this subfamily of Leguminosae they are a constant feature even at depths of 4 feet, and seem normally to last as long as the epidermis. It was impossible to determine the

nature of the contents, if any, because of the dense walls of the hairs whose chemistry was not investigated. The suggestion was offered that the persistence of root hairs, as well as of thorns, might be an archaic condition of a xerophytic type now adapted to various habitats.

WHITAKER (16) investigated a number of genera of the Compositae as well as certain of the Leguminosae and came to the general conclusion that there is a definite correlation between a permanent hirsute condition and lack of secondary thickening. It was intimated that such hairs might be functional, since she stipulated that the radial arrangement of the primary root stele was adapted to absorption whereas the concentric disposal of the secondary one was not. Another casual statement was of interest; namely, that the exodermis in contrast to the endodermis was probably a fairly new and rather inconstant feature of root organization, but occurred constantly in the species studied by her. It is the opinion of the present writer that this exodermis is definitely associated with the production of secondary hairs as will be proposed in a subsequent paragraph.

A brief comment upon WHITAKER's paper by POPESCO (12) may be translated: "WHITAKER describes some plants which never produce secondary tissues in their roots and which absorb just the same; she names them apilose." Apparently POPESCO had not grasped the meaning of her term which signified simply a condition where root hairs were lacking. On the persistent feature he made no comment, nor did he mention it in his own research except indirectly that in *Sambucus nigra* there were alternating zones of young and old hairs, proving that the hairs were not absorptive but had "quite another function" which he did not discuss. ARBER (1) has stressed the peculiarity of the typical monocotyledonous root associated with the lack of secondary thickening and absence of deep-seated periderm. She states that the root hairs were surrounded by jacket cells and contained delicate simple or branched rods of highly refractive substances at their bases. It is not clear what she meant by the former, but perhaps they would correspond to the atrichoblastic cells of LEAVITT. She also defines the exodermis as a generally cutinized layer broken by passage cells and occurring below the piliferous one.

MILLER (11) states: "The length of life of root hairs depends on the species of the plant and upon the moisture conditions of the soil. Root hairs of some species live for only a few days or weeks, while those of others exist for several years, although they do not function in absorption for a period that long."

Thus it appears that although thick-walled root hairs have been noted for some time, the general assumption has been that they are merely persistent primary hairs whose function has not been satisfactorily explained.

Material and methods

The following served as research material:

COMMELINACEAE.—*Tradescantia edwardsiana* Tharp, *T. geniculata* Jacq., *T. gigantea* Rose, *T. hirsuticaulis* Small, *T. hirsutiflora* Bush, *T. occidentalis* Smyth, *T. paludosa* Anderson and Woodson, *T. reverchoni* Bush, *Setcreasea brevifolia* (Torr.) Rose, *Palisota barteri* Hook., *P. bracteosa* C. B. Clarke, *P. mannii* C. B. Clarke, *Dichorisandra thyrsiflora* Mikan, *Commelina coelestis* Willd., *C. communis* L., *Tinantia fugax* Scheidw., *Rhoeo discolor* Hance, *Zebrina pendula* Schnizl.

OTHER MONOCOTYLEDONS.—*Philodendron cordatum* Kunth, *Asparagus officinalis* L., *Zea mays* L.

The root material was studied in regard to the superficial aspect, the internal anatomy, and the physiology.

SUPERFICIAL ASPECT

A number of species were examined from herbarium specimens with regard to color, extent, approximate length, and tenacity of the root hairs. Small cuttings of *Rhoeo discolor* and *Dichorisandra thyrsiflora* were grown in a water chamber fitted on the stage of a microscope, so that a fresh solution of tap water was kept flowing continuously (apparatus copied after FARR, 3). Cuttings of mature *Rhoeo discolor* had been taken from the soil and immersed in water to produce usable rootlets within a week's time. The cuttings of *Dichorisandra thyrsiflora* were obtained from plants being cultivated in water.

Anatomy

The specimens were examined in the living state, both superficially and as freehand sections, and also as preserved material. Most of the latter was killed in chrom-acetic acid or Jeffrey's picric acid-corrosive sublimate reagent, dehydrated through Zirkle's butyl alcohol series, imbedded in paraffin and cut at 10 μ. Celloidin was used as an alternative matrix and proved superior for keeping the hairs intact. Safranin and light-green was the most effective stain combination for paraffin sections, whereas iron-alum haematoxylin served for the celloidin. In order to determine the nature of the root hair walls, microchemical tests were made with chloriodide of zinc as a test for cutin, and KOH plus the latter for suberin. Various stains were also used, especially safranin, Congo red, and Magdala red. The tests employed by Scott (**14**) were tried also.

Physiology

A cutting of *Setcreasea brevifolia* which had been growing in tap water for a number of days and had produced a root of several centimeters was fitted into the water chamber. This was attached to a 5 liter flask from which a solution of methylene blue (1:1,000,000) was suctioned off. The rate of flow was adjusted by a stopcock so that the solution was exhausted in about 12 hours; it was then re-employed for the length of the experiment, 36 hours. The whole apparatus was kept in a dark room with an even temperature and good ventilation. The purpose of this procedure was to attempt a vital staining in order to note contents and activities of the hairs.

An attempt was made to follow Popesco's (**12**) method with iron sulphate and potassium ferrocyanide. His description was indefinite as to whether ferric or ferrous sulphate was utilized and as to the amounts and the time. Dilutions of 1:10,000 ferrous sulphate employed overnight gave the best results in the present experiment. Cuttings of *Commelina communis* grown in tap water for several days were immersed in the ferrous sulphate solution in three series: those normal and intact; those incised in the zone of older hairs and immersed directly; and those incised as above and sealed with melted vaseline.

Results

Superficial aspect

Externally the majority of the Commelinaceae have roots which are slender but somewhat succulent, and more or less completely and luxuriantly hirsute from near the tip to approximately the top. Often the lower centimeter or so is hyaline and the rest of a dark brown or occasionally white tomentum. The former condition is typical of *Rhoeo discolor*, the primary experimental plant. The species of *Palisota* and *Dichorisandra* show a similar condition, the delimitation of white and brown hairs being conspicuous even in the soil. The roots of *Tradescantia reverchoni* are very hirsute. These plants are of tropical habitat but widely planted in greenhouses. *T. hirsuticaulis* has peculiar little drop roots thickened at the distal ends from which protrude tiny rootlets. This type of root, rather common in the monocotyledons, is especially associated with bulbous plants and has a contractile function (1). *T. edwardsiana*, *T. gigantea*, and *T. occidentalis*, found in moist fertile soils of the Mississippi Valley, have essentially the same type of piliferous layer. *T. paludosa*, a lesser plant with smaller roots, grows in drier regions and has longer hairs associated with a slightly different anatomical structure. *Zebrina pendula* shows a minimum amount of hirsuteness even in water-cultivated specimens, and the roots are small for experimental purposes.

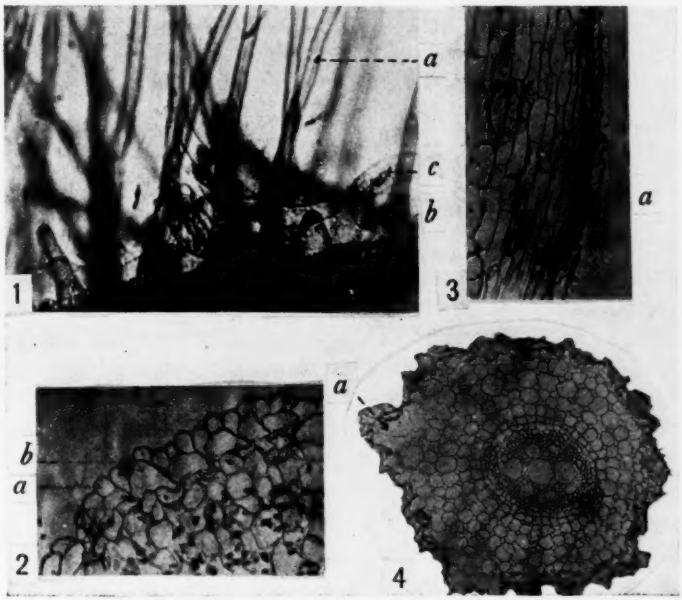
Cuttings of *Commelina communis* grown in water develop white hairs under the surface and brown ones on all exposed parts above it, except for the uppermost centimeter or so which is glabrous.

The roots of *Philodendron cordatum* have a brown tomentum externally not unlike most of the species of *Tradescantia*. *Asparagus officinalis* is very hirsute and *Zea mays* to a lesser degree.

The use of external root hair features as delimiting characters in taxonomy seems feasible, since there is a constancy in this regard in plants otherwise showing close relationships.

Microscopic observations of a specimen of *Rhoeo discolor* growing in the water chamber first revealed the origin of the brown hairs. Microscopically there seems to be an abrupt change from hyaline to brown hairs, but magnification portrays a narrow transition zone

where among the long slender transparent hairs the short russet thicker ones are noticeable (fig. 1). The latter are of secondary origin, that is, they come from the exodermis and supersede the primary hairs. They arise usually several centimeters from the tip in the region of older epidermal hairs. The latter, together with the cres-



FIGS. 1–4.*—Fig. 1, origin of secondary hairs in *Rhoeo discolor*, ×450: *a*, primary hair; *b*, secondary hair; *c*, displaced epidermal (atrichoblastic) cell. Fig. 2, enlarging hypodermis in *Tradescantia paludosa*, ×100: *a*, epidermal cell; *b*, hypodermal cell pushing aside epidermis. Fig. 3, longitudinal section showing origin of secondary hairs in *Philodendron cordatum*, ×100: *a*, hypodermal cell becoming piliferous. Fig. 4, cross section of mature root of *Rhoeo discolor*, ×30: *a*, origin of secondary hair.

* All drawings made with an Abbé camera ludica at ×700. Reduction approximately two and one-half times.

cent atrichoblastic cells (9), are pushed aside as the new ones emerge from the exodermis. From the outset they are sturdier than their predecessors and their heavy walls effectively mask any contents. Seedlings of *Tinantia fugax*, *Commelina coelestis*, and *Tradescantia*

geniculata germinated on filter paper revealed the presence of secondary hairs at a young stage when the rootlets were only several centimeters long.

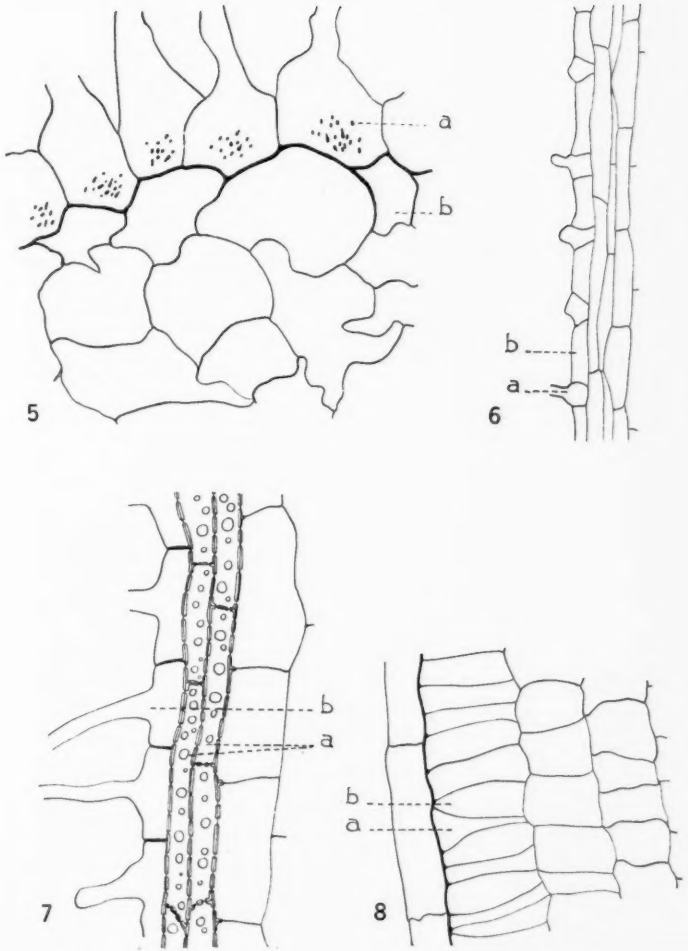
No attempt was made to correlate data from the dicotyledonous roots, but those cursorily examined from a number of garden plants showed only epidermal hairs. Seedlings of lettuce germinated in a moist chamber indicate, however, that new hairs may arise at various indiscriminate places in the piliferous layer, which is contrary to the general view that they are formed only in acropetal succession.

ANATOMY

Most of the roots of the Commelinaceae display the following structural features: a relatively small stele, usually polyarch, with several large lacunae in the pith and with no secondary growth; a thin-walled endodermis, often detectable in cross section only by Casparian strips; a prominent turgid cortex whose cells are amply supplied with plastids and starch grains and surrounded by large air spaces; an exodermis of one to several layers with walls thickened on the three outer sides, excepting the passage cells; and a piliferous layer (fig. 2). In the oldest parts of the roots of *Commelina communis* there are no hairs. Internally the endodermis has gone to the tertiary stage (become completely suberized), so there is no interchange of material between cortex and stele. The roots of *Philodendron cordatum* are similar to the preceding, except for the distinction of mucilage canals and isolated tracheids in the cortex (fig. 3). *Asparagus officinalis* has several layers of cylindrical exodermal cells (fig. 7).

Cell contents, including nuclei, are visible in stained secondary hairs of fresh material at a distance of several centimeters from the tip. The walls also appear irregularly thickened internally. The heavy-walled hairs give the test for cutin. In *Dichorisandra thyrsiflora* the inner walls of the piliferous layer are lined with tiny granules which stain red with safranin (fig. 5). This phenomenon was noted also by ARBER (1).

The trichoblastic-atrichoblastic condition is well shown in *Palisota barteri* (fig. 6). In *Tradescantia gigantea* the exodermis is delimited at an early age. The conspicuous exodermal layer of large



FIGS. 5–8.—Fig. 5, *Dichorisandra thyrsiflora: a*, refractive granules; *b*, cutinized hypodermal cell. Fig. 6, *Palisota barteri: a*, trichoblast; *b*, atrichoblast. Fig. 7, thickened pitted hypodermal cells in *Asparagus officinalis: a*, hypodermal cell; *b*, epidermal cell. Fig. 8, differentiation under root cap in *Tradescantia gigantea: a*, trichoblast; *b*, atrichoblast.

protruding cells in *T. paludosa* shows lateral expansions (fig. 2). Further development of the cortical hair initials appears in *Rhoeo discolor* and in *Philodendron cordatum* (fig. 4).

Physiology

The few experiments tried with dyes were not convincing. After 30 hours in weak methylene blue solution, cuttings of *Setcreasea brevifolia* exhibited root tips colored blue for a distance of half a centimeter. Sections showed staining mainly in the stele, although there was some in the cortex and an indeterminate condition in the brown hairs.

Results following Popesco's (12) technique were unsatisfactory also; the stele and the piliferous layer were stained but not the cortex.

The brown hairs of *Rhoeo discolor* themselves have a fuzzy appearance owing to the small particles of undetermined substances clinging thereto, which may be formed by chemical precipitation and indicate absorption. Their walls are thickened and not gelatinous like those of the primary hairs.

Discussion

The conclusion of Jeffrey and Torrey (7) and Whitaker (16), that the occurrence of "persistent hairs" is associated with the lack of secondary growth, applies to those monocotyledons where secondary thickening is known only infrequently. But the fact that the hairs most certainly arise secondarily and are obviously of physical structure different from the primary ones is puzzling. Since their walls are thickened, as well as those of the cells lying immediately underneath, absorptive ability is made doubtful in spite of passage cells in the exodermis. On the other hand the observed contents (including nuclei, external adherent particles), the turgid cortex, and primary endodermis, suggest that absorption may be possible.

Anchorage may be another function of secondary root hairs, as several investigators have suggested. The facts that these plants have a comparatively shallow root system and often grow in open soil, as well as that there is a tenacious adherence of soil particles,

further this hypothesis. In one case, *Tradescantia hirsuticaulis*, the epidermal layer is obviously specialized for contraction.

If, as WHITAKER (**16**) stipulates, the exodermis is a rather new feature of root organization, its constant presence in these fundamental groups of monocotyledons suggests a relatively late origin, concomitant with that of the herbaceous Compositae and Ranunculaceae, at a time when perhaps environmental conditions were such that an expansive surface of hairs was for some reason desirable.

LEAVITT'S (**9**) exclusive segregation of the monocotyledons with trichoblastic layers and dicotyledons with transfusion layers does not seem justified. In certain representatives of the former, the writer finds that the original hairs are of trichoblastic nature and predetermined in the young part of the root under the root cap (fig. 8). The secondary hairs, in the same, arise from exodermal cells (transfusion layer); and, as shown in *Palisota barteri*, apparently the trichoblastic condition repeats itself (fig. 6).

Summary

In the members of the monocotyledons investigated there exists a so-called "persistent" hirsuteness of practically the whole root system. The constituent cutinized hairs have been found to be of secondary cortical origin, superseding the primary epidermal ones. It seems logical that this condition may be associated with the lack of secondary thickening, but the function has not been determined.

Appreciation is due DR. R. E. WOODSON, JR., who suggested this problem and assisted its progress, and DR. G. T. MOORE for the use of the facilities of the Missouri Botanical Garden.

111 North 41st Street
OMAHA, NEBRASKA

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MICROSPOROGENESIS AND CYTOKINESIS IN ASIMINA TRILOBA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 474

JOHN FLOWERS LOCKE

(WITH PLATE I)

Introduction

The North American pawpaw, *Asimina triloba* (L.) Dunal, occurs throughout nearly the whole of the eastern half of the United States, exclusive of the New England states.

Little cytological work has been reported on the two genera of the Anonaceae, *Asimina* and *Anona*, which occur in the United States. VOIGT (**8**) investigated the structure and development of the peculiarly ruminated endosperm in *Uvaria lowii*, and stated that the other members of the family show no marked differences in either structure or development. NICOLOSI-RONCATI (**4, 5**) reported on the formation of the endosperm and the structure of the ovule in *Anona cherimolia*. HERMS (**1**) described a few stages in the early development of the megagametophyte in *Asimina triloba*. SAMUELSSON (**7**) reported that microspore formation in *Aristolochia clematitis* is by successive constriction, that the same type of constriction occurs in *Asimina*, and discussed the systematic significance of such a type of microspore formation.

Since none of these reports deal with meiosis and the number of chromosomes, a study has been made of nuclear behavior during meiosis in *Asimina triloba*. On examining the material of *Asimina*, a peculiar type of cytokinesis was noticed; and since the heterotypic prophases of the meiotic divisions showed little of significance, this investigation has concerned itself mainly with the type of cytokinesis occurring in the divisions of the pollen mother cells in the formation of microspores.

Materials and methods

Material of *Asimina* was collected in the spring of 1931 near Mississippi State College and in 1933 and 1934 in the region of Smith,

Indiana. Most of the material was killed in the field. The first step in the preparation of the material was to remove the sepals and petals from the floral buds. Good penetration was obtained when the buds were dropped in Carnoy's solution for a few seconds before being placed in the fixing solution. For the earlier stages good results were obtained by the use of a suction pump immediately following immersion of the buds in the fixing solution.

Several fixing agents were used, among them various modifications of Navashin's solution, Flemming's medium and stronger solutions, chromo-acetic-osmic solution, Carnoy's solution, and corrosive sublimate. The modifications of Navashin's solution gave the most satisfactory results.

Sections were cut from 4 to 12 μ, those about 6 μ proving most satisfactory for the study of both nuclear and cytoplasmic division. The preparations used for the study of meiosis were stained in iron-alum haematoxylin, while those used in the study of cytokinesis were counterstained with orange G. Owing to the strong affinity of the mother cell wall for the crystal violet, Flemming's triple stain proved unsatisfactory for preparations designed to show cell division.

Observations

Resting nucleus to diakinesis

In the resting condition the pollen mother cells are polyhedral in shape. Usually two pollen mother cells, separated by a single layer of sterile cells, occur across the diameter of the locule. The nuclei now have a diameter of approximately 8 μ. The strands of the reticulum are thin, and where they cross and anastomose, the chromatin is aggregated in thick masses. There is usually one large deeply staining nucleolus, rarely two of unequal size.

As the prophases begin, the nuclei of the mother cells enlarge rapidly, the chromatin network becomes more threadlike, the aggregations spread out along the threads, and a smoother and more uniform appearance is gradually assumed. Soon after these changes occur the threads pair side by side.

The synizetic condition now ensues; but, contrary to the usual condition, in which the synizetic knot lies on the side of the nucleus closest to the mother cell wall, in *Asimina triloba* the knot is on the

side of the nucleus farthest from the wall. The nucleolus is commonly not in contact with the synizetic knot, but lies free within the nuclear cavity. It is always spherical at this time, and may show small peripherally arranged vacuoles. During the changes just described the nuclei have enlarged gradually and continue to do so until at pachytene they have a diameter of approximately 16 μ.

Following synizesis, the chromatin threads, consisting of double strands closely twisted about each other, begin to spread throughout the nuclear cavity. In some cases this association was so close that their double nature could not be detected, but at other places the twisting of the strands was clearly evident.

As the pachytene condition begins, the threadlike mass continues to loosen and spreads throughout the entire nuclear cavity. During early pachytene there is little evidence of the double nature of the strands; but as they pass into diplotene, the paired elements become less closely associated. The threads continue to shorten and assume the typical form and scattered arrangement of bivalent chromosomes which characterize diakinesis. Counts made of bivalent chromosomes at diakinesis indicated a haploid number of nine, although it is not possible to make a definite statement concerning their number. During the anaphases of the second meiotic division nine chromosomes were observed passing to the poles of the two daughter cells, thus tending to confirm this number as being the correct count.

Cytokinesis

First division.—Following diakinesis, the mother cell wall continues to thicken uniformly until at the time the microspores are formed it has a thickness of approximately 6 μ. The spherical pollen mother cells lie free in the anther locule. During diakinesis, the nuclear membrane disappears and the fibers of the diarch spindle, which appears at about this time, become attached to the chromosomes. As the chromosomes pass toward the poles, the mother cell becomes elongated in the direction of the axis of the first division. Occasionally the mother cells begin elongation before the end of the prophases. During interkinesis, the chromatin material of the daughter nuclei does not become reorganized into a definite reticu-

lum, but assumes the form of spherical, irregular, or threadlike masses (figs. 1–5, 7–9). The daughter nuclei are discoid and usually somewhat flattened on the side toward the region of the first cytoplasmic constriction (figs. 3–5, 7–9). After the daughter nuclei have been formed, the spindle spreads out until it extends almost across the mid-plane of the cell (fig. 2). The spindle fibers are thicker and more irregularly arranged than during the anaphases; and as cytokinesis occurs, they gradually become more granular.

The first evidence of cytokinesis is the appearance of a constriction in the equatorial region and in a plane approximately equidistant from the daughter nuclei (fig. 2). The furrow is usually initiated after the daughter nuclei have been formed; in some cells, however, the furrow is initiated during the anaphases of the first meiotic division. When the furrow first begins to form it is narrow and pointed, but soon thickens considerably (fig. 3). The advancing edge of the furrow becomes broad and round, giving the cell a two-lobed appearance, and from the time it is initiated it is completely filled with material in close apposition to the mother cell wall.

In no cell observed was there any indication of a cell plate prior to the initiation of the furrow; but when a cytoplasmic isthmus of approximately one-third the diameter of the mother cell remains between the advancing edges of the furrow, an inconspicuous cell plate may be formed in the mid-region of the spindle (figs. 3–5). This condition occurred in a number of the cells observed in this stage of development; in others there was no indication of such a structure.

The furrow continues to deepen; and when the isthmus remaining is about one-fifth the diameter of the mother cell in the direction of the constriction, the cell plate, if formed, has completely disappeared (figs. 6–8). Thus the cell plate formed here is an evanescent structure and plays no part in the furrowing process. The spindle fibers have now disappeared also, and nothing was observed to indicate that they play a part in the formation of the developing plasma membranes, as suggested by FARR (2).

Within a short time the cytoplasmic isthmus is completely severed by the constriction of the plasma membrane, and the wall of the

mother cell forms a continuous structure between the two daughter cells (fig. 9). The wall which develops between the daughter cells is about three times as thick as the mother cell wall surrounding the two cells and, as shown by microchemical tests, consists of callose.

Since there is no definite correspondence between nuclear phases and stages in the furrowing process, it is not possible to arrange a series of stages in cytokinesis on the basis of nuclear changes. In the majority of cells the first cytoplasmic division is completed before the second nuclear divisions occur, although occasionally the latter may occur during the process of the first constriction.

SECOND DIVISION.—The spindles of the second nuclear division figures now form and are usually parallel with one another and perpendicular to the axis of the spindle of the first division. Figure 10 shows a cell during the anaphases of the second nuclear division with nine chromosomes passing to each pole. Following this the spindles disappear, and no suggestion of a cell plate was observed in any of these division figures.

The furrow of the second cytoplasmic division begins along the inner periphery of the daughter cells adjacent to the wall of the first division (fig. 11). It proceeds to about one-third the diameter of the cells and now begins to appear along the outer peripheral wall (fig. 12). Furrowing then proceeds on all sides at about the same rate, and constriction is completed at a point closer to the outer peripheral wall than to the wall which comes in following the first constriction furrow (fig. 13). In figure 14 a condition is shown in which one of the daughter cells is dividing more rapidly from the outer wall, and constriction of one of the daughter cells will be completed closer to the wall of the first division than to the mother cell wall. Usually the second divisions are completed at approximately the same time, but occasionally one of the daughter cells may divide before the other. One of the daughter cells may undergo the second cytoplasmic division before second nuclear division has been initiated in the sister cell (fig. 15).

The two successive divisions of the pollen mother cell form four microspores which are quadrilaterally arranged (fig. 16). The locules of the anther are of such small diameter that one quartet of

microspores fills the entire space. After the second division is complete, the nuclei lie close to the plasma membrane of the microspores, but not in contact with it. After a time they become centrally placed.

Development of pollen grain

The young microspores, with dense granular cytoplasm, measure approximately 30 μ in diameter (fig. 16). They soon begin to enlarge, apparently with little increase, if any, in the amount of cytoplasm at this time. With the increase in size the vacuoles enlarge and the cytoplasm remains for a time distributed throughout the cell. At the time the microspore has reached its full size, the cytoplasm lies mainly at one side, having one or a few large vacuoles in the central portion of the cell (fig. 17). During enlargement of the microspore its wall increases in thickness, developing a pectose intine layer and an exine layer of undetermined composition. After a time the cytoplasm begins to increase in volume; simultaneously the vacuoles become smaller and more numerous, until the cytoplasm of the microspores finally appears densely alveolar (figs. 18–21).

The nucleus of the microspore now divides, thereby forming the generative and the tube nuclei of the young microgametophyte (fig. 21). This division may occur before the cytoplasm has become densely alveolar (fig. 20). The generative nucleus is smaller than the tube nucleus, and it, with a small amount of cytoplasm, is set off from the tube cell by a membrane. When the binucleate condition of the microgametophyte occurs the tapetal tissue has disappeared and the anthers have opened for the shedding of pollen.

Discussion

Farr (2) gives an extensive review of the literature dealing with cytokinesis in pollen mother cells. His results tend to indicate that the divisions of the pollen mother cells, in most angiosperms, are brought about by furrowing rather than by cell plates. In general, bipartition is common to the monocotyledons and quadripartition to the dicotyledons, but intermediate types of microspore formation have been reported for a few scattered forms. Some of those which are similar to *Asimina* will be discussed briefly.

ROSANOFF, in his work on *Acacia paradoxa*, gives two figures (**6**, figs. 24, 25) showing bipartition of the pollen mother cells by furrowing. After completion of the first division, the furrowing appears along the periphery of the daughter cells and later along the wall of the first division furrow. Cell plates are not shown, and he does not discuss the process of cytokinesis. From these two figures it appears that the division in *A. paradoxa* is similar to that which occurs in *Asimina triloba*. It differs, however, in that in the former the furrow of the first division appears first along the outer periphery of the daughter cells, while in *Asimina* it appears first along the inner periphery of the daughter cells adjacent to the wall of the first division furrow.

In his study of *Magnolia*, FARR (**3**) reported that the first cytoplasmic division of the pollen mother cells begins during interkinesis and continues until the isthmus of cytoplasm which remains is about one-half the diameter of the mother cell. Division is now arrested, the fibers disappear, and the second nuclear divisions occur. During interkinesis, the furrow of the first division may almost disappear. The furrow of the second division appears at the periphery of the cell and at right angles to the axis of the spindle of the first division. The spindle of the first nuclear division has now reappeared. The furrow of the second division deepens more rapidly than the furrow of the first division so that both constrictions are completed at approximately the same time. There was no indication of a true cell plate after the initiation of the constriction. FARR suggested that furrowing might have been brought about by attraction between the nuclear and cytoplasmic membranes.

FARR (**2**) observed that the spindle fibers come in contact with the plasma membrane at the advancing edge of the furrow, and suggested the possibility of their being used in the formation of the developing plasma membranes. The spindle fibers in *Asimina* were not observed to come in contact with the plasma membrane as the furrow advanced.

SAMUELSSON (**7**) reported a type of cytokinesis in *Anona cherimolia* that is similar to that in *Asimina triloba*. Division is by successive constriction, and the mother cell is divided into two daughter cells. The furrow begins during the telophases of the first nuclear

division and is completed after the second nuclear divisions have occurred. During the metaphases of the second nuclear division, the cells are connected by a narrow bridge of cytoplasm. The second division takes place in the same manner as the first. *Asimina* differs from *Anona* in that there is not so close a correlation between nuclear phases and stages in the furrowing process in the former as occurs in the latter. The second division in *Asimina* differs from the second division in *Anona* in that (as already shown) it does not agree completely with the first division as SAMUELSSON described for *Anona*. SAMUELSSON thought that the type of division that occurred in *Anona* was more closely related to the monocotyledonous than to the dicotyledonous type.

SAMUELSSON reported a similar type of division in *Aristolochia clematitis*. Immediately after the first cytoplasmic division the intervening wall thickens greatly. This was observed to be true in *Asimina*. In *Aristolochia* the spindles of the second division may not always be parallel to one another and perpendicular to the axis of the spindle of the first division. The four microspores may be arranged in a straight line as a result of the spindles of the second division being in the same plane as the spindle of the first division. In some cells he reported one of the spindles of the second division to be parallel with the spindle of the first division and the other perpendicular to it, the two spindles of the second division being in the same plane.

HERMS observed the pollen mother cells in *Asimina triloba* to be differentiated in early October, and stated that they remain in this condition until the middle of January. He reported that on March 10 the pollen mother cells begin to enlarge, and by April 14 a bridge of tissue is formed between the microsporocytes. Spore tetrads are formed by April 21. By May 5 pollen is ready to be shed. Similar definite dates were given for the stages in development of the endosperm and megagametophyte. My own observations indicate that it is not possible to associate stages of development with precise dates because of the great variation in the different buds used for study. Floral buds were collected in late March in which pollen mother cells had not become differentiated. Also, in some collected as late as May 1, microspores had not been formed.

Summary

1. During meiosis the chromatin material of the pollen mother cells undergoes parasynaptic association.

2. At synizesis the chromatic knot lies on the side of the nucleus farthest from the mother cell wall.

3. The haploid number of chromosomes is apparently nine.

4. Cytokinesis of the pollen mother cells is by successive constriction. Evanescent cell plates may occur in the first division.

5. In general the first cytoplasmic division is completed before the second nuclear divisions occur.

6. In the second cytoplasmic division the furrow appears first on the side of the daughter cells adjacent to the wall of the first division, and proceeds outward for a time before it appears at the outer periphery of the cell.

7. The young microspores are relatively small and have a quadrilateral arrangement as a result of the spindles of the second division being perpendicular to the axis of the first division and in the same plane with it.

8. Before being shed, the microspores develop into relatively large binucleate microgametophytes which are completely filled with dense, alveola-like cytoplasm.

The writer expresses appreciation to Dr. J. M. BEAL for suggestions and constructive criticism throughout the course of this investigation.

DEPARTMENT OF BOTANY
MISSISSIPPI STATE COLLEGE
STATE COLLEGE, MISSISSIPPI

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EXPLANATION OF PLATE I

FIG. 1.—Pollen mother cells showing first nuclear division.

FIGS. 2–9.—Pollen mother cells showing successive stages in first cytoplasmic division.

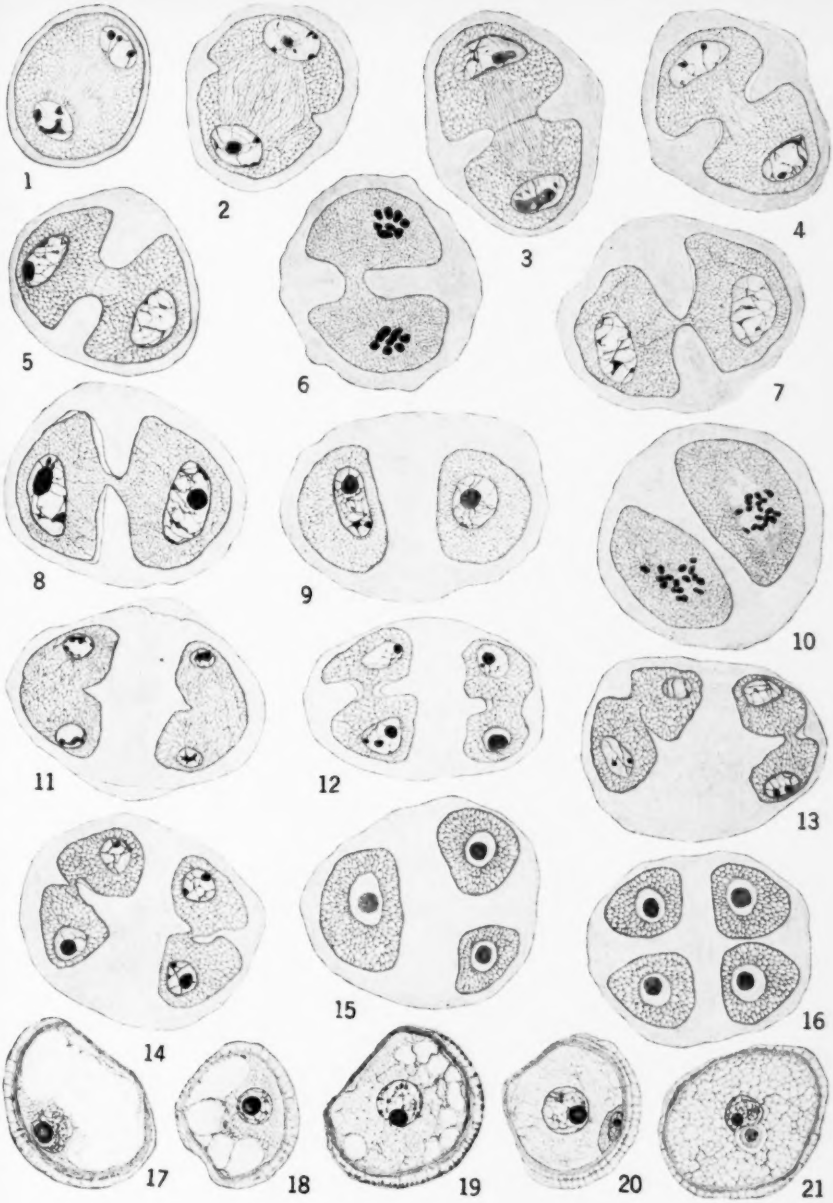
FIG. 10.—Daughter cells in early anaphases of second nuclear divisions with nine chromosomes passing to each pole.

FIGS. 11–14.—Daughter cells showing successive stages in second cytoplasmic division. Fig. 14, unusual condition in one daughter cell in which the furrow has proceeded more rapidly from outer periphery toward wall of first division.

FIG. 15.—One daughter cell which has undergone second cytoplasmic division before the second nuclear division of sister cell has been initiated.

FIG. 16.—Quartet of microspores quadrilaterally arranged.

FIGS. 17–21.—Successive stages in development of pollen grain from mature microspore.



LOCKE on ASIMINA

DEVELOPMENT OF THE MALE GAMETES OF LILIUM[1]

D. C. COOPER

(WITH THIRTY-ONE FIGURES)

Introduction

Differing observations have been made regarding the history of the male gametes in the Liliaceae, and especially in the genus *Lilium*. The present investigation was undertaken because the findings of certain recent investigators are almost diametrically opposed to those of earlier workers. An attempt has been made to get a series of stages, from generative cell to fertilization, in the development of the male gametes of *Lilium regale* Wilson. For comparative purposes, material of *L. auratum* Lindl. and *L. philippinense* Baker was also studied.

MATERIALS AND METHODS.—In order to obtain stages of the division within the generative cell, stigmas of *Lilium regale* and of *L. auratum* were pollinated and the material (stigma and style) collected at intervals between 48 and 72 hours after pollination. Some of these stigmas and styles were split lengthwise, fixed in Karpechenko's modification of Navashin's fluid, imbedded in paraffin, sectioned, and stained to show the pollen tubes in the stylar canal. In other cases the pollen tubes were gently scooped from the stylar canal on to a clean slide and fixed for one hour in a modification of Carnoy's solution (95 per cent alcohol, 6 parts; glacial acetic acid, 2 parts; chloroform, 2 parts). These were then washed in 95 per cent alcohol and mounted in aceto-carmine.

Slides bearing pollen tubes were also placed in Karpechenko's fixing fluid, washed and stained by the iodine-crystal violet-picric acid method. When the extremely delicate pollen tubes were removed from the stylar canal, care was taken not to pull or stretch them.

[1] Paper no. 198 from the Department of Genetics, Wisconsin Agricultural Experiment Station. Published with the approval of the Director of the Station.

Another method of obtaining pollen tubes was to germinate the pollen grains on a slide. The sticky exudate of the stigma was smeared on a clean slide and pollen dusted thereon. Such slides were placed in a moist chamber for 36 to 48 hours, then fixed in Karpechenko's fluid and stained either in Delafield's haematoxylin or by the iodine-crystal violet process. Pollen of *Lilium regale*, *L. auratum*, and *L. philippinense* was thus germinated and treated.

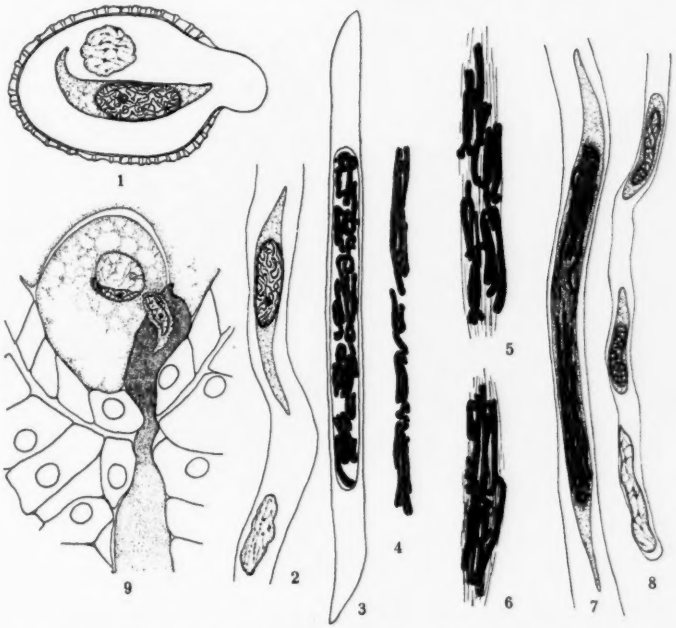
Observations

Lilium regale.—The pollen tube appears at a fissure of the ruptured exine (fig. 1) and grows downward between the papillate hairs of the stigma until it reaches the epidermis. It then bends toward and enters the stylar canal and grows along the canal toward the ovary.

The nucleus of the tube cell usually precedes the generative cell into the pollen tube (fig. 2), although it may lie beside or follow the generative cell. The tube nucleus is irregular in shape and structure (figs. 2, 8). When it lies beside the generative cell it is usually drawn out into a dumb-bell shape in consequence of the small diameter of the pollen tube.

At the time of germination of the pollen grain, the nucleus of the generative cell is in an early prophase stage (fig. 1). This cell and its nucleus become markedly elongate upon entering the pollen tube (fig. 2). During the period in which the pollen tube is growing along the stigmatic surface and down the funnel-like upper end of the stylar canal, the deeply staining chromosomes are formed, each of which shows a longitudinal split (figs. 3, 10). Shortly after the pollen tube and the included generative cell have passed into the narrow neck of the stylar canal (from 48 to 60 hours after pollination), the nuclear membrane disappears (figs. 5, 6, 12–15). A typical spindle is present. The figures are elongated because of the length of the chromosomes and the small diameter of the pollen tube. The longitudinally split chromosomes divide on the equatorial plate (figs. 16, 17). Figures 7 and 18 show late anaphase stages with twelve chromosomes at each pole. A cell plate is formed across the mid-region of the spindle, and two separate gametes (cells) are formed. The nuclear material of each daughter nucleus spreads out into a

chromatic network approximating that of a so-called resting stage (figs. 8, 9, 19). The zone of cytoplasm about each male gamete nucleus persists until fertilization. Figure 9 shows a stage in fertilization, the male gamete nucleus near the egg nucleus having now escaped from its cytoplasmic sheath, since no such sheath can be

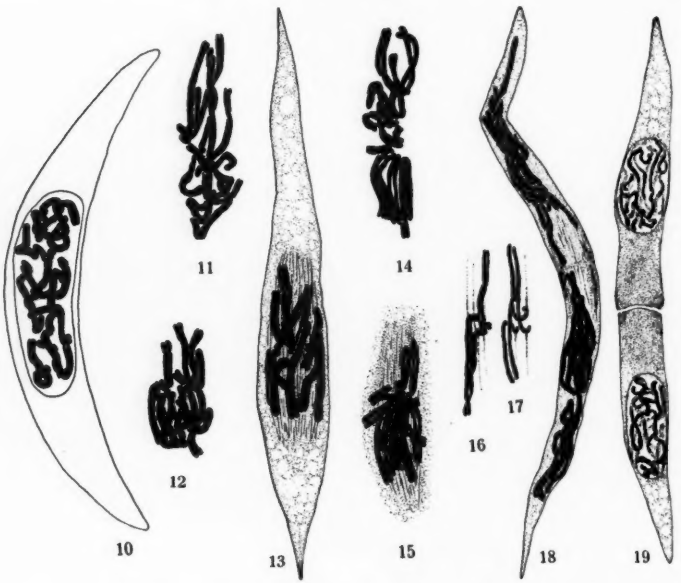


FIGS. 1–9.—*Lilium regale*, stages in division of generative cell as found in pollen tubes growing in stylar canal: Fig. 1, germinating pollen grain; ×400. Fig. 2, generative cell and tube nucleus in pollen tube; ×400. Fig. 3, generative cell; nucleus in late prophase stage of division; ×780. Fig. 4, chromosome advancing to equatorial plate; ×780. Fig. 5, early metaphase; ×780. Fig. 6, metaphase; ×780. Fig. 7, late anaphase showing early stage of cell plate formation; ×780. Fig. 8, tip of pollen tube containing tube nucleus and two male gametes; ×400. Fig. 9, fertilization; one male gamete nucleus near egg nucleus, second leaving ruptured pollen tube; ×780.

discerned. The second male gamete (still a complete cell with nucleus and cytoplasm) is just leaving the ruptured pollen tube.

When the pollen tubes are grown in culture, the generative cell

(figs. 10–17) divides in a manner very similar to that just described. The cell and nucleus are not so elongated, however, and the diameter of the pollen tube is somewhat greater than when it grows in the stylar canal. The chromosomes are shorter and thicker in culture, but under either condition of growth they have similar



FIGS. 10–19.—*Lilium regale*, stages in division of generative cell as found in pollen tubes grown in culture: Fig. 10, generative cell; nucleus at late prophase stage; ×780. Figs. 11, 12, chromosomes advancing to equatorial plate; ×780. Fig. 13, early metaphase; ×780. Figs. 14, 15, metaphase; ×780. Fig. 16, three chromosomes at metaphase; ×780. Fig. 17, two chromosomes at early anaphase; ×780. Fig. 18, late anaphase showing cell plate formation; ×780. Fig. 19, two male gametes; cell plate completed; ×780.

morphological features. As seen in the equatorial plate, they vary in length so that the longest chromosome is approximately 2.5 times as long as the shortest. Three of the chromosomes have median or submedian spindle fiber attachments; nine have subterminal attachments (fig. 5). In these respects the appearances are similar to those

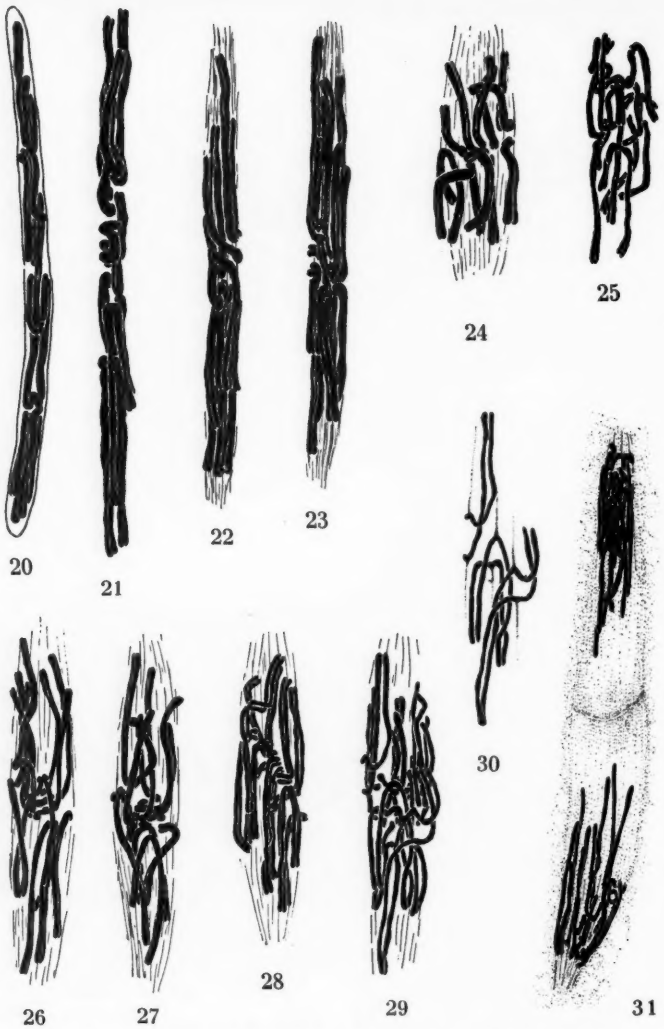
seen in the haploid divisions in the formation of the embryo sac of *L. henryi* (1). A somewhat shorter spindle occurs in the material grown in culture (figs. 13–15) as compared with material found in the stylar canal. Cell plate formation (fig. 18) likewise occurs and two male gamete cells are formed (fig. 19).

Lilium auratum.—The division of the generative nucleus in this species resembles that described for *L. regale.* The chromosomes of the elongated nucleus (fig. 20) as found in pollen tubes located in the stylar canal are drawn into an equatorial plate (figs. 21, 22), and the division figure is typical, a well formed spindle being present (figs. 22–23). A late prophase (fig. 20) shows the longitudinally split chromosomes, which retain their double nature until the halves separate in the equatorial plate. In those pollen tubes grown on the stigmatic exudate in a moist chamber, the chromosomes are shorter and thicker and the spindle is more definite (fig. 24).

Lilium philippinense.—The material of this species examined was from pollen tubes grown in culture. Figure 25 shows the chromosome complement of the generative nucleus shortly after the dissolution of the surrounding membrane. These chromosomes pass on to a typical mitotic spindle (figs. 26–28). At the equatorial plate stage (fig. 28) the spindle fiber attachment regions are approximately in the equatorial plane of the spindle. Each chromosome begins to divide at the spindle fiber attachment region (fig. 29). This is clearly evident in figure 30, showing three of the chromosomes of the complex in the preceding figure. Two of these chromosomes have approximately median spindle fiber attachments; one has a subterminal attachment. In the anaphase (fig. 31) the chromosomes are elongated and the median and subterminal spindle fiber attachments are clearly evident. In the cell represented in this figure a cell plate is being formed.

Discussion

The method of division of the generative nucleus and generative cell of the three species of *Lilium* herein described is in agreement with the observations of a number of investigators of other members of the Liliaceae [*Lilium martagon* (**2, 4, 8**); *L. candidum* (**3**); *Hemerocallis flava* (**9**)]. These observations are at variance with those of



FIGS. 20–31.—Stages in division of generative cell taken from pollen tubes growing in stylar canal. *Lilium auratum:* Fig. 20, late prophase; fig. 21, chromosomes advancing to equatorial plate; fig. 22, early metaphase; fig. 23, late metaphase; figs. 24–31, stages in same division as found in pollen tubes growing in culture; fig. 24, metaphase. *L. philippinense:* Fig. 25, chromosomes advancing to equatorial plate; figs. 26, 27, early metaphases; fig. 28, late metaphase; fig. 29, early anaphase; fig. 30, three of chromosomes of fig. 28 showing spindle fiber attachments; fig. 31, late anaphase showing cell plate formation. All ×1025.

certain other workers who found no signs of a spindle in this division. NAVASHIN (**5**) observed no stage in *L. martagon* which might be interpreted as a typical metaphase. In the earlier stages of division, however, he observed longitudinally split chromosomes. His figures 7 and 14 are interpreted as equatorial plate stages. They are similar to the late prophases of *L. regale* and *L. auratum* shown in figures 4 and 21 respectively, where the chromosomes are advancing to the equatorial plate. Since NAVASHIN saw no spindle fibers, he concluded that the chromosomes pass to the telophase position by some type of movement peculiar to themselves.

WELSFORD (**10**) also saw no spindle at any stage of this division in either *Lilium auratum* or *L. martagon*. She found the division process similar in these two species and described it for *L. martagon* in detail. Her figure 16, which approximates an equatorial plate stage, and figure 17, also entitled an equatorial plate stage, are likewise similar to my figures 4 and 21. TRANKOWSKI (**9**) observed a metaphase without a spindle in *Convallaria majalis*.

O'MARA (**6**) examined the corresponding division in *Lilium regale* and concluded that a typical equatorial plate is lacking since he could not demonstrate the presence of spindle fibers. An examination of his figures shows that most of his observations are based on configurations similar to my figures 4, 5, and 11, which must be interpreted as late prophases rather than as equatorial plate stages (figs. 6, 13–15, 22–24, 26–29). That these are late prophase configurations is all the more probable when it is considered that his material was collected between 24 and 36 hours after pollination, whereas the material showing equatorial plate stages in the present study was collected 48 to 72 hours after pollination. In the case of pollen germinated in culture, the stages shown in figures 10, 11, and 12 were obtained from material fixed after 36 hours in the moist chamber, while abundant configurations showing equatorial plates, anaphases, and telophases were present in cultures which were kept in a moist atmosphere from 42 to 48 hours. O'MARA's figures 2, 4, 12, and 13, termed metaphases, are similar to my figures of stages at which the chromosomes are passing to the equatorial plate. His figure 8 approximates the late prophase stage shown in figure 5 of *L. regale* and in figure 22 of *L. auratum*. In the three species of

Lilium here discussed, the division of the generative nucleus appears to be regular.

Differing observations have been made as to the nature of the male gametes of the Liliaceae. GUIGNARD (2) observed the male gametes to be distinct cells in *L. martagon*, whereas both KOERNICKE (4) and STRASBURGER (8) found only male gamete nuclei in that species. STRASBURGER described the formation of an evanescent cell plate which disintegrates, leaving naked gamete nuclei in the cytoplasm of the pollen tube. WELSFORD (10) in *L. auratum* and *L. martagon*, and HERRIG (3) in *L. candidum*, showed that the male gametes exist as distinct cells in these species. WELSFORD found that the male gametes lose their cytoplasm upon entering the embryo sac. TRANKOWSKI (9) reported division of the generative cell to be by constriction in *Convallaria majalis;* the cytoplasm of each male gamete, according to him, soon disappears, leaving naked nuclei. O'MARA (6) found some evidence of constriction in this division in *L. regale*. According to him, the gamete cytoplasm dissolves and at later stages two naked nuclei are present in the cytoplasm of the pollen tube. The division of the generative cell is by means of cell plate formation in *Lilium regale* and *L. philippinense*. In all three species studied the male gametes exist as distinct cells. In the case of *L. regale*, the sheath of cytoplasm around each male gamete nucleus was observed to persist until fertilization.

Varying observations have been made in other families of plants regarding the nature of the division of the generative cell and the type of male gamete present (9). Mitotic figures have been described in the generative cell and many cases are known in which the male gametes exist as distinct cells.

Summary

1. The nucleus of the generative cell in *Lilium regale*, *L. auratum*, and *L. philippinense* passes through a typical mitotic division in the production of the two male gametes.

2. A distinct spindle is present.

3. Three of the chromosomes have median or submedian spindle fiber attachments; the other nine chromosomes have subterminal attachments.

4. The generative cell is divided by means of a cell plate.
5. The male gametes are fully organized cells.

DEPARTMENT OF GENETICS
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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ANATOMICAL STUDY OF THE SEEDLING OF HIBISCUS TRIONUM

ANNA MAY KIMMELL

(WITH TWENTY-FIVE FIGURES)

Introduction

A study of *Hibiscus trionum* L., which is a common weed in the state of Nebraska and elsewhere, was made to determine the details of root-stem transition. Some work has been done on related species. GERARD (3) and THOMAS (8), working with *Althaea rosea*, described the normal seedling as typical of the "Anemarrhena" group; but they also found that the seedling might exhibit considerable variation in its method of transition. In 1926 BEXON (1) continued this work.

MATERIALS AND METHODS.—The plants were grown from seeds collected in the fall and winter at Ames and Lincoln, Nebraska.

Formalin-acetic-alcohol was used as fixing agent for the various stages of development. Each seedling was cut into segments approximately 7 mm. long in order that the fixative might thoroughly penetrate the tissues. The material remained in the fixative at least 10 days before dehydration. Safranin and gentian violet were used as stains. Freehand sections of a great number of seedlings were cut to determine the typical symmetry. These were dehydrated in alcohol and mounted in sandarac. Whole seedlings were cleared in cedar oil.

GERMINATION.—To overcome the difficulty in germinating seeds in the usual way, several methods were tried. Cutting the seed coat with a sharp razor blade resulted in abnormal seedlings. Freezing and burning were found to be unsatisfactory, but treating the seeds with concentrated sulphuric acid for 20 minutes resulted in seedlings which were perfect in all respects (fig. 1). Seeds left in acid for 25 and 30 minutes had 100 per cent germination but did not develop normally. In 48 hours, 16 per cent exhibited a maximum length of 14 mm. The average length was 5 mm. and the minimum was 0.5

mm. These seedlings were undoubtedly injured by the penetration of the acid into the embryos. The radicles appeared stunted and there was no development of root hairs.

Investigation

Seedlings of three different ages were used: 5, 16, and 24 days old. The root-stem transition could be interpreted best in the 5-day seed-

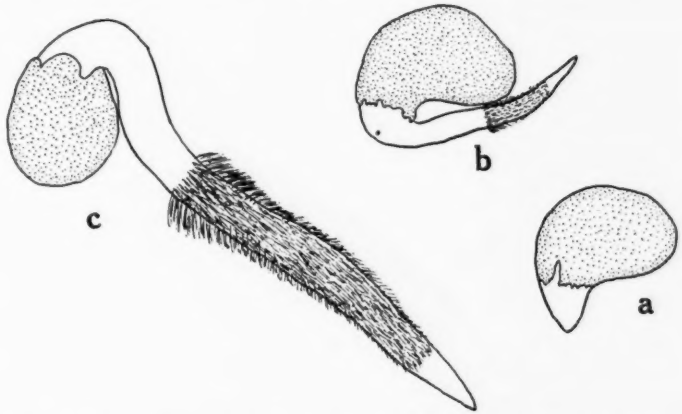


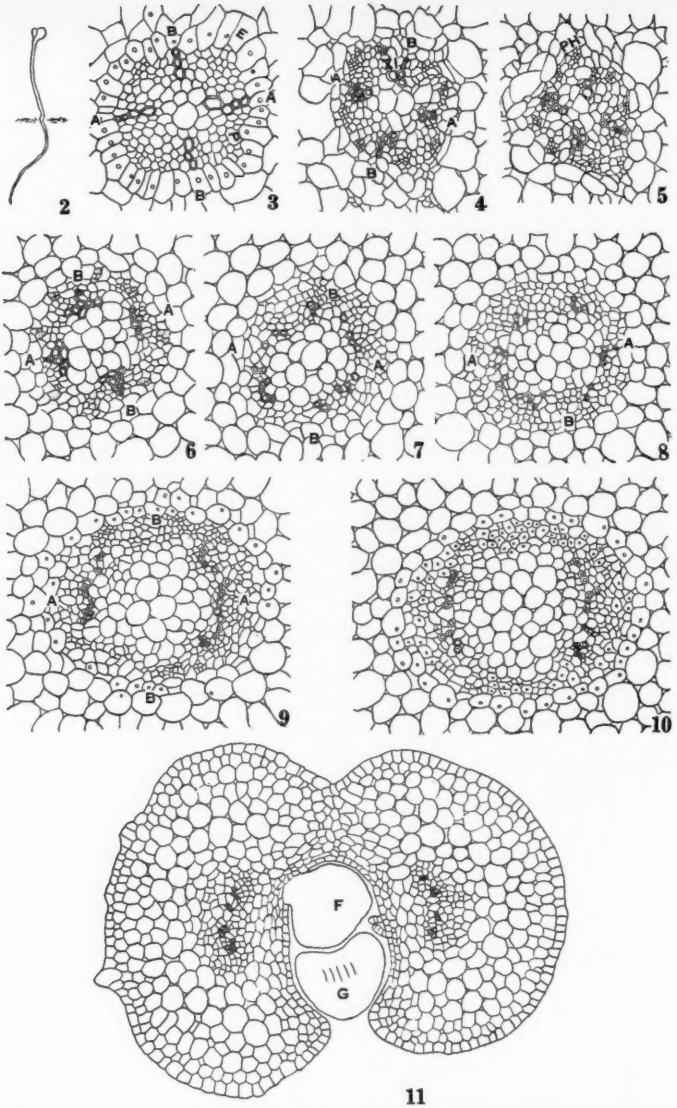
FIG. 1.—Germinating seeds in various stages of development: *a*, radicle emerging from seed coat; *b*, root 5 mm. long with root hairs; *c*, seed with root 13 mm. long, root hairs abundant.

lings. Older seedlings were used to study the stem structure and the vascular system of the first leaves.

From stem tip to root tip a 5-day seedling measures approximately 50 mm. (fig. 2). The two cotyledons are then completely unfolded, one of the laminae being slightly larger than the other, but the first foliage leaf is not visible. The hypocotyl is covered with many stellate and glandular hairs.

The root is tetrarch (fig. 3). The pericycle (*P*) is intersected at four points where the protoxylem abuts the endodermis. The cells of the endodermis vary from the radially elongated ones (*E*, fig. 3) to irregular cells that are difficult to follow in transverse section (figs. 4, 5). The cortex is from seven to ten cell layers in thickness.

Sections at slightly higher levels (figs. 4, 5) show that two phloem



FIGS. 2–11.—Figs. 2–10, drawings of 5-day-old plant and from serial sections through root and hypocotyl (*A-A*, cotyledonary plane; *B-B*, intercotyledonary plane): fig. 2, 5-day-old seedling ($\times\frac{1}{2}$); fig. 3, tetrarch stage; fig. 4, eight phloem groups; fig. 5, endodermis irregular; fig. 6, differentiation of metaxylem forming xylem arcs; figs. 7, 8, differentiation of each arc into two or more distinct groups; figs. 9, 10, intercotyledonary plane conspicuous *B-B*. $\times$100. Fig. 11, sections cut slightly above cotyledonary node of 5-day-old plant (*F*, bud with meristematic cells; *G*, petiole of first foliage leaf). $\times$100.

groups (*PH*) have developed in each angle formed by the xylem rays.

The "xylem intermediary" (*XI*) as described by CHAUVEAUD (2) is present at this level (figs. 4, 5). At a higher level (fig. 6) the metaxylem elements are differentiated in the shape of arcs. Some of the protoxylem vessels in the radial position are distorted and partially disintegrated.

During the subsequent processes of growth, some of the protoxylem elements become obliterated and the compact arcs of the metaxylem (fig. 6) change in appearance, each arc becoming separated into two more or less distinct groups (figs. 7, 8). At this level the intercotyledonary plane cannot be determined. Soon the vascular elements form an interrupted oval (fig. 9) and growth in the intercotyledonary plane (*B-B*) divides the elements of the two intercotyledonary polar groups. Half of the vascular tissue of each goes to each cotyledonary plane, and there occupies a lateral position with respect to the polar protoxylem elements of that plane. The xylem elements at this level are largely protoxylem, consisting of vessels with annular and spiral thickenings. There are only a few tracheids.

Because of the reorientation of vascular tissues in the transition region, the xylem in the cotyledonary plane, near the point of divergence of the cotyledonary petioles, is made up of the polar or main xylem group of that plane and a part of each of the polar xylem groups of the intercotyledonary plane. Owing to the disappearance of some polar protoxylem elements and the addition of the portions of the two intercotyledonary polar xylem groups, the cotyledonary trace consists of four distinct groups of xylem (fig. 10). In the cotyledonary trace each of these xylem groups lies internal to the phloem group so that the bundles are collateral. As such a cotyledon is separated from the cotyledonary node, a line of cleavage in the parenchyma is formed. The cotyledons show the alternate phyllotaxy which is characteristic of this plant. In a given transverse section the vessels of one cotyledon are seen in transverse section while the vessels of the other appear in longitudinal section. This is also evident externally at the cotyledonary node (fig. 11), as one petiole is larger than the other.

The entire vascular system of the cotyledons is continuous with the primary vascular system of the root. The cotyledonary trace consists of the "double bundle" and two laterals. The "double bundle," first described by THOMAS (7), is comparable with her second type which is sometimes represented by two distinct bundles which together constitute one pole of the root. In addition to the two central bundles, there is frequently present a lateral bundle on each side, so that there may be four bundles in each cotyledonary trace. The condition found here is similar to that described by THOMAS (7) for *Althaea*.

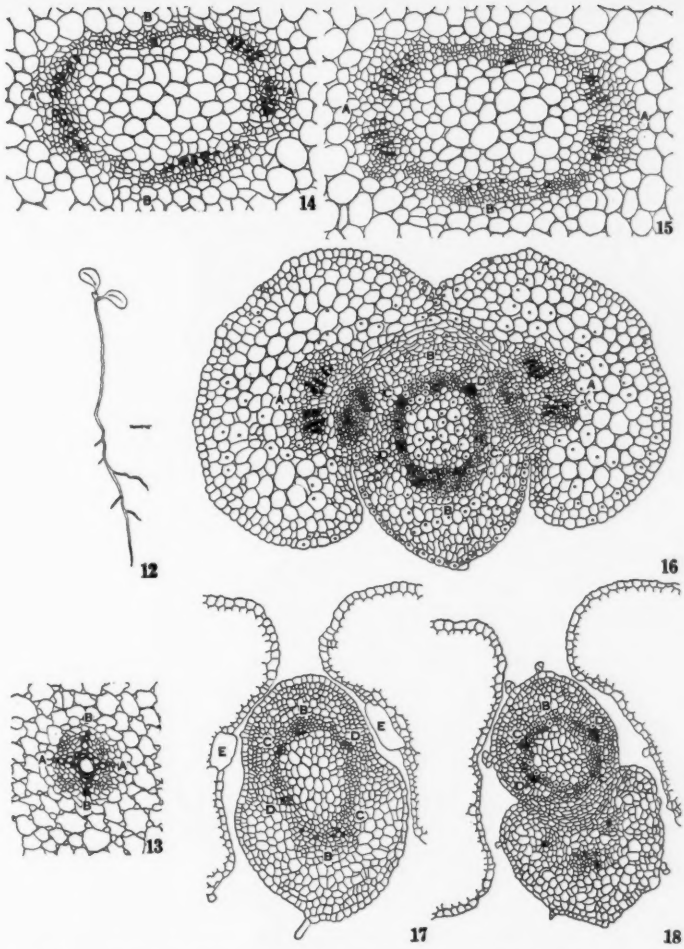
The bud apex of the epicotyl (*F*) lies between the petioles of the two cotyledons. On the intercotyledonary side of this stem meristem the first foliage leaf is differentiated as a protuberance of tissue. In this rounded mass of tissue are differentiated rows of radial procambial strands which form the trace of the first leaf. As the petiole of this leaf is diverged from the epicotyl, the undifferentiated meristem (*G*) may be seen in the center, arranged in linear rows (fig. 11).

DIFFERENTIATION OF FIRST AND SECOND LEAVES

Seedlings 16 days old measured approximately 100 mm. from root tip to stem tip.

In addition to the primary xylem elements of the cotyledonary plane, vessels which have developed from the intercotyledonary meristem, so prominent in figure 10, are evident (figs. 14, 15). On one side are a number of vessels, while on the other there are only two (fig. 14). The meristematic ring of the hypocotyl becomes more and more elliptical in shape, owing to extensive growth in the intercotyledonary plane (fig. 15). As the first cotyledonary petiole is cut off, the meristematic cells of both intercotyledonary planes are united on that side. The parenchyma cells in the cotyledonary gap are meristematic and form a bud (*E*) in the axis of the cotyledonary petiole (figs. 16, 17).

At this stage the meristem in the region of the epicotyl is horseshoe-shaped, but is again cylindrical above the point of divergence of the second cotyledonary petiole from the main stem (fig. 16). A bud is formed in this cotyledonary gap (figs. 16, 17). At a higher



FIGS. 12–18.—Figs. 12–15, 16-day-old plant (×½) and drawings from series of transverse sections: fig. 13, protoxylem and metaxylem in tetrarch root; figs. 14, 15, vessels of epicotyl in intercotyledonary plane *B-B*. ×100. Fig. 16, 16-day-old plant, transverse section at cotyledonary node showing petioles of cotyledons and epicotyl with its continuous meristematic ring. ×70. Figs. 17, 18, transverse sections through epicotyl (*C-C*, diagonal plane; *D-D*, diagonal plane; *E-E*, meristematic buds in cotyledonary axis): fig. 17, vessels in intercotyledonary plane *B-B*, meristematic cells with denser contents in diagonal planes upper *C* and *D-D*; fig. 18, petiole of first foliage leaf distinct from epicotyl. ×70.

level the meristematic ring of the epicotyl changes from an oval (fig. 16) to an ellipse (fig. 17), which is oriented with its long axis at right angles to the cotyledonary plane.

The uppermost portion of the 16-day-old seedling shows the differentiation of the first two leaves, and the structure of the epicotyl indicates where the third leaf will develop.

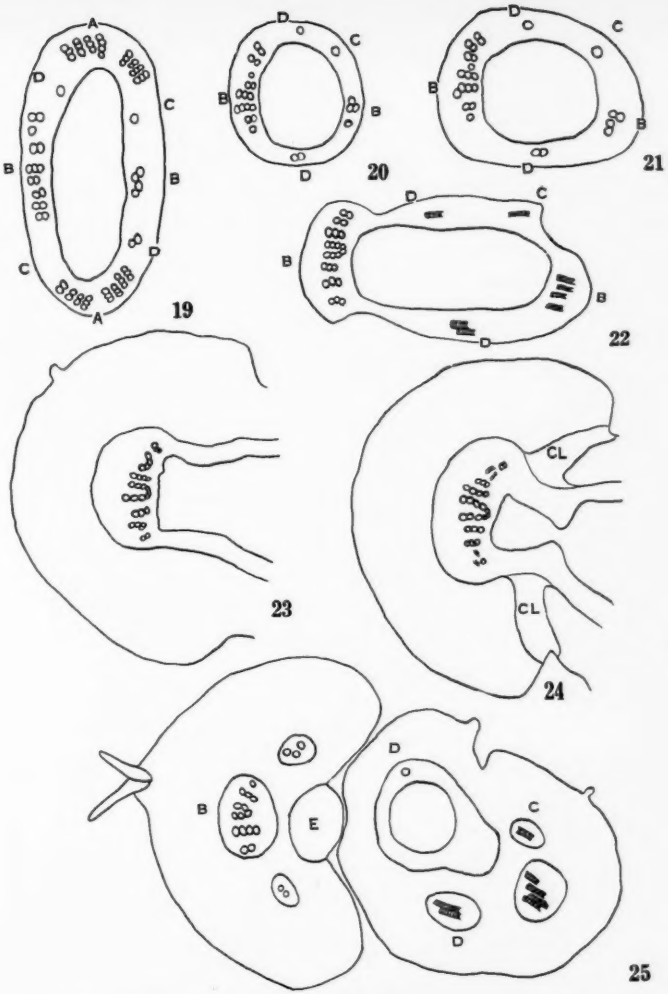
The vessels which are continuous with the first foliage leaf trace are shown in lower *B*, figure 17. The one or two vessels opposite this xylem arc in the intercotyledonary plane are continuous with the midrib of the second foliage leaf (upper *B*, fig. 17). The protuberant tissue of the epicotyl, which later diverges as the petiole to the first leaf, is visible (*B*, fig. 18) as it was in the younger seedling. At the level where the first leaf is diverged from the epicotyl, two conspicuous groups of cells occur (*C* and upper *D*, fig. 18) in the meristematic ring farthest from the petiole. These are continuous with the laterals of the second foliage leaf.

Development of leaf traces

When the seedling is 24 days old the origin, development, and termination of the first foliar elements may be followed.

At the cotyledonary node xylem elements differentiate in the intercotyledonary plane (fig. 19, *B-B*) and are directly continuous with the elements of the hypocotyl. These vessels in the intercotyledonary plane are more numerous on one side than on the other, and this condition is correlated with the phyllotaxy. At the cotyledonary node one xylem vessel occurs on one side of the cotyledonary arcs of vessels (upper *C* and *D*, fig. 19) while on the opposite side two vessels are differentiated (lower *D*, fig. 19). The meristem of the epicotyl is continuous and oval in shape as in the younger seedlings (fig. 20). In this ring, which becomes more and more elliptical at successively higher levels, there are now four vessels (*B*, fig. 21) instead of two as shown in figure 18. Regions *D* and *C* each have one vessel, and lower *D* has two instead of the meristematic groups that occurred in the 16-day seedling.

At this level the arc of vessels (fig. 22) that is continuous with the first leaf is oriented in transectional view, while the vessels that are continuous with the traces of the second and third leaves appear in



FIGS. 19–25.—Figs. 19–22, diagrams showing meristematic ring and vessels of 24-day-old seedling (*A-A*, cotyledonary plane; *B-B*, intercotyledonary plane; *C-C* and *D-D*, diagonal planes): fig. 19, section at hypocotyl showing cotyledonary vessels *A-A*, which become traces of first and second foliage leaves *B-B*, and vessels in diagonal planes *C* and *D-D;* figs. 20–21, vessels of epicotyl; fig. 22, vessels which become traces of first foliage leaf differentiating in vertical plane, opposite them the vessels which become traces of midrib of second foliage differentiating in horizontal plane and adjacent vessels also differentiating in horizontal plane. Figs. 23, 24, transverse sections through petiole of first leaf; fig. 23, vessels intact; fig. 24, a few vessels on either side of intact group; *CL*, region where cleavage will take place. Fig. 25, transverse section of 24-day-old plant showing petiole of first foliage leaf distinct with midrib and two laterals; vessels in diagonal planes *C-D* differentiating to form laterals to second leaf; meristematic ring complete with vessel *D* which is continuous with midrib of petiole of third leaf.

longisection. The groups of vessels situated at each end of the arc (*B*, figs. 23, 24, 25) form the laterals of the first leaf.

The four vessels (right *B*, fig. 22) and the adjacent vessels (*C* and lower *D*, fig. 22) are continuous with midrib and lateral traces of the second leaf (fig. 25). The protuberance of the epicotyl (fig. 25) contains three groups of vessels and at a higher level there is a cleavage line in the parenchymatous tissue where the petiole of the second leaf is diverged. In the ring of meristematic tissue is seen one vessel (upper *D*, fig. 25) which is one of those continuous with the midrib of the third leaf.

Discussion

A paper by SILER (5) thoroughly reviews the history of the work on root-stem transition. This paper stresses CHAUVEAUD'S (2) theory in contrast to that of VAN TIEGHEM (9). The transition stages observed in *Hibiscus trionum* are in agreement with CHAUVEAUD'S theory.

The type of transition found in *Hibiscus trionum* differed from that found in *Thespesea* by SINNOTT (6). SINNOTT'S diagrams indicate that at the cotyledonary node there are two collateral bundles in each cotyledonary petiole, and six bundles in the region of the epicotyl. The bundles of the cotyledonary petiole vary from two at the point of petiolar attachment to four at the base of the cotyledonary blade.

H. trionum deviated from this method. From the typical radial, tetrarch root, two phloem groups appeared between each of the four xylem arcs. At the cotyledonary node there were four xylem groups in each cotyledonary petiole. These were formed from the cotyledonary xylem appearing as two groups owing to the obliteration of the protoxylem elements of the cotyledonary poles and from the division of the xylem rays of the intercotyledonary plane. This indicates that the entire primary vascular system of the root is continuous with that of the cotyledons. After the divergence of the cotyledonary petioles, the lateral groups of meristem in the intercotyledonary plane become continuous. This process is repeated after the separation of each succeeding petiole. These meristematic cylinders occurring repeatedly are procambial tissue.

SINNOTT (6) states, "By far the most common condition at the cotyledonary node is that shown in *Thespesia populnea*, *Momordica balsamina*, and *Lavatera arborea* where the bundles of the epicotyl arise entirely in the intercotyledonary plane and the traces of each cotyledon make but a single gap in the vascular ring." He further states that this corresponds to the unilacunar nodal type in the mature stem. *H. trionum* might also be added to this list, because of its conformity in regard to the bundles of the epicotyl arising in the intercotyledonary plane as well as to the fact that the traces of each cotyledon make but a single gap in the vascular cylinder.

The "double bundle" found in the petiole of the cotyledons of *H. trionum* is comparable with THOMAS' (7) second type of "double bundle." She states, "it was found that the 'double bundle' is sometimes represented by two quite separate bundles (*Ricinus*, *Quercus*, etc.). . . . . The function of these central bundles, whether they appear as two widely separate entities, or so closely approximated as to have the appearance of a single strand, is always the same, *viz.*, to form between them one pole of the root." She also says, "in addition to the two central bundles or their 'double bundle' equivalent, there is frequently present a lateral bundle on each side of them, so that there are four bundles in each cotyledon (*Ricinus*, *Quercus*, *Fagus*) or the equivalent of four (*Casuarina*, *Polygonatum*, *Crataegus*, *Acacia*, *Medicago*, *Althaea*, etc.)." As *Althaea* is one of the Malvaceae and a close relative of *Hibiscus*, this correlation between the two is to be expected.

THOMAS (7) also states, "when the lateral bundles are large and important looking, they penetrate into the hypocotyl and unite with the corresponding bundle from the opposite cotyledon to form the alternating poles of a tetrarch root." This is precisely the condition found and carried out in the root-stem transition of *H. trionum*.

Summary

1. The anatomy and transition stages from root through hypocotyl of 5-day-old seedlings of *Hibiscus trionum* were studied; the vascular anatomy of the epicotyl of 16-day-old and 24-day-old plants was also examined.

2. The differentiation of "intermediary" vessels, and the disap-

pearance of some protoxylem elements in the root and hypocotyl, cause each of the four radial xylem rays to appear in the shape of an arc.

3. Obliteration of the protoxylem elements and further differentiation in the hypocotyledonary region result in the rearrangement of the vessels of each arc into two more or less distinct groups.

4. The intercotyledonary plane is made up of active meristematic cells in the 5-day-old seedling.

5. The xylem in the cotyledonary plane is formed from the polar xylem group and a part of each of the lateral groups.

6. The cotyledonary trace is made up of a "double bundle" and two laterals.

7. The vessels that differentiate in the intercotyledonary plane of the 16- and 24-day-old seedlings form the traces to the first and second leaves which occur at alternate nodes. Only one element of the third leaf had been differentiated in the 24-day-old seedling.

8. The vessels constituting the two arcs in the intercotyledonary plane vary greatly in number.

9. The lateral vessels in the petiole of the first leaf are formed from the intact arc of vessels in that petiole.

10. The lateral vessels in the petiole of the second leaf arise in a longitudinal diagonal plane in the hypocotyl region.

11. The cotyledonary node is elliptical in the cotyledonary plane while the second and third nodes are elliptical in the opposite plane. The fourth and fifth nodes are elliptical again in a plane above and parallel with the cotyledonary plane.

12. The recurring meristematic ring is procambium.

I wish to express my gratitude to Dr. EMMA N. ANDERSEN whose assistance has made this work possible and to Dr. ELDA R. WALKER for many helpful suggestions.

KIMMELL, INDIANA

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DEVELOPMENT OF THE MACROGAMETOPHYTE IN CERTAIN LABIATAE

ELIZABETH P. BUSHNELL

(WITH TWENTY-TWO FIGURES)

Introduction

SCHNARF (4, 5) has summarized the observations upon the formation of the nucellus, integument, archesporium, and macrospores in the Labiatae. The ovule is anatropous, with a single thick integument. The archesporial cell of the nucellus is subepidermal; it functions as a macrospore mother cell. Of the four macrospores formed by its division, the innermost develops into a seven-celled, eight-nucleate gametophyte. The antipodal cells disintegrate in most cases before fertilization. The polar nuclei fuse in the chalazal part of the gametophyte. Endosperm formation follows one of four somewhat diverse types in the various genera.

RUTTLE (2, 3) has traced the history of the macrogametophytes of *Mentha* and *Lycopus*. Minor differences occur in species of *Mentha* and *Lycopus* in size of macrospores, time of development of the tapetal layer, shape of gametophyte, time of fusion of the polar nuclei, and the presence or absence of an obturator.

In *Monarda fistulosa* and *Nepeta cataria*, BILLINGS (1) describes a large coenocytic micropylar haustorium containing a few relatively small nuclei.

Material and methods

Development of the macrogametophyte has been studied in *Monarda fistulosa* L., *M. didyma* L., *M. punctata* L., and *Nepeta cataria* L. Most of the material used was collected in the Pharmaceutical Gardens of the University of Wisconsin; some of the material of *M. punctata* came from plants growing in sandy fields near Pine Bluff, Wisconsin, and some of *M. didyma* from private gardens in the vicinity of Madison.

Flower buds and individual flowers were fixed in Karpechenko's, Navashin's, Licent's, Flemming's medium, and Carnoy's acetic-

alcohol-chloroform solutions. Best results were obtained with Navashin's, Licent's, and Flemming's fluids. Fixed material after the usual treatment was imbedded in paraffin. Sections were cut from 10 to 15 μ in thickness and stained with Flemming's triple stain or with Heidenhain's iron-alum haematoxylin.

All figures were drawn with a camera lucida at table level. A Leitz microscope with a $\frac{1}{16}$ inch, 1.32 N.A. oil immersion objective, and 3×, 4×, 8×, and 12× oculars was used.

Observations

Monarda fistulosa

When microspore mother cells are first recognizable in the anther, the ovule (one in each ovary lobe) appears as a slight protuberance from the base of the sporangial chamber. It curves as it grows (fig. 1), finally becoming anatropous. The nucellus consists of one layer of cells.

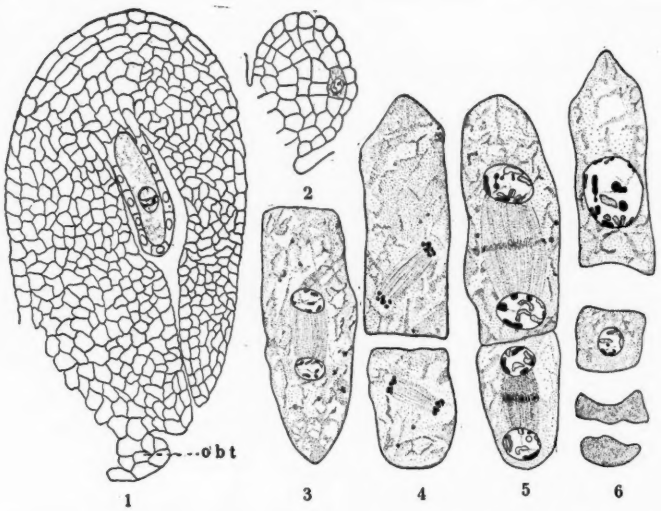
An archesporial cell, differentiated before the single integument shows much indication of development (fig. 2), functions directly as a macrospore mother cell. In some instances the integument has surrounded the nucellus when the nucleus of the macrospore mother cell is still in a presynizetic stage. Macrospore mother cells with nuclei in synizesis, however, were observed in other ovules whose integument incloses the nucellus by the time of formation of the heterotypic equatorial plate.

When the single integument has grown about half way around the nucellus, a projection (the obturator, fig. 1) appears below the ovule. When the ovule is fully developed, the massive obturator extends around the micropyle.

Two nuclear and cell divisions (figs. 3, 4) result in the formation of a typical row of four macrospores. Both cell divisions are brought about by means of cell plates (fig. 5). Degeneration of the macrospores is invariably from the micropyle backward; the large spore at the chalazal end is the one that functions (fig. 6). This macrospore enlarges and large vacuoles appear above and below the nucleus. After nuclear division, the two daughter nuclei lie in the central region of the macrogametophyte; however, they soon move apart (fig. 7) and the macrogametophyte elongates and broadens. A large

vacuole forms in the central part, smaller vacuoles now being present at the ends. The large vacuole also characterizes the four-nucleate stage (fig. 8), but is no longer present at the eight-nucleate stage (fig. 9).

The mature seven-celled gametophyte is broad at its micropylar end; the remainder is narrow and tubelike (fig. 10), curving some-

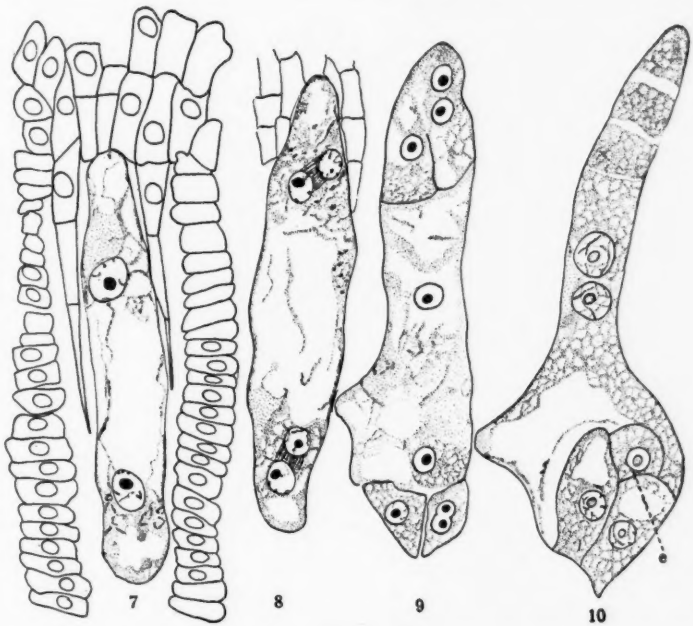


FIGS. 1–6.—Macrogametophyte development in *Monarda fistulosa:* Fig. 1, ovule showing single integument and obturator (*obt*); macrospore mother cell surrounded by one-layered nucellus. Fig. 2, young ovule showing archesporial cell. Fig. 3, telophase, heterotypic division. Fig. 4, anaphase, homoeotypic division. Fig. 5, cell-plate formation following homoeotypic division. Fig. 6, chalazal macrospore persisting, other three macrospores disintegrating. Figs. 1, 2, ×315; all others, ×1025.

what toward the vascular bundle of the ovule. The egg, as it grows, becomes differentiated into a stalk, in contact with the macrogametophyte wall, which contains little stainable cytoplasm, and an enlarged spherical portion with rather densely staining cytoplasm. The synergids are long and narrow. On the sides nearest the periphery of the macrogametophyte each synergid develops a ridge. The effect of a partial cap is produced, as described by RUTTLE (2) for *Mentha requienii*. The antipodals may sometimes be seen disinte-

grating before the two polar nuclei have fused. Fusion of these nuclei occurs before fertilization in the central part of the cell.

At the period of the heterotypic diakinesis, nucellar cells nearest the micropyle appear somewhat crushed and stain darkly. Lateral nucellar cells at the two-nucleate stage of the macrogametophyte

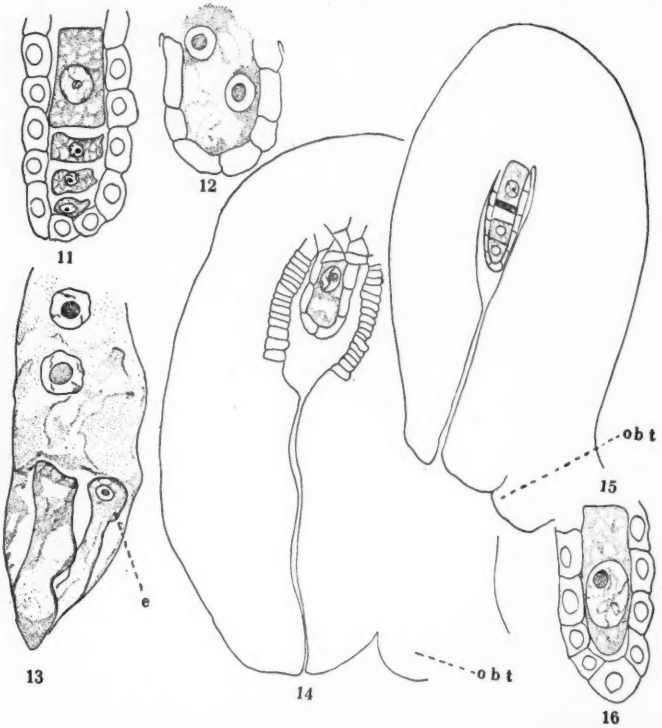


FIGS. 7–10.—Macrogametophyte development in *Monarda fistulosa:* Fig. 7, two-nucleate gametophyte, a few chalazal nucellar cells, "tapetum," and hypostase. Fig. 8, four-nucleate macrogametophyte. Fig. 9, eight-nucleate macrogametophyte, cell division in progress. Fig. 10, macrogametophyte showing egg (*e*), synergids, and two polar nuclei; antipodal cells already disintegrated. ×785.

now also take a dark stain and begin to disintegrate. As the two-nucleate gametophyte grows, it pushes out through the nucellar epithelium and crushed nucellar cells are then digested. Only a few cells in the chalazal region of the nucellus persist during the entire period of development of the macrogametophyte.

Cells in the chalazal portion of the integument become modified

to form the hypostase tissue. At the time when there is an axial row of macrospores, the hypostase consists of only a few chalazal cells which stain darkly. When the gametophyte is one-nucleate, the walls of the hypostase cells are thickened. The hypostase is well de-

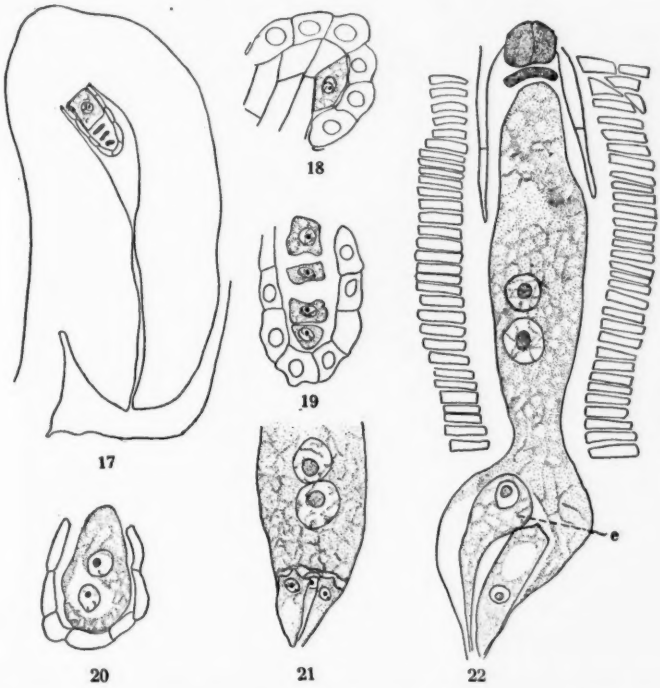


FIGS. 11–16.—Macrogametophyte development in *Monarda didyma* (figs. 11–14) and in *M. punctata* (figs. 15, 16): Fig. 11, four macrospores, the chalazal the largest. Fig. 12, nucellus with two-nucleate macrogametophyte. Fig. 13, egg (*e*), one synergid, and polar nuclei. Fig. 14, ovule with massive integument, large micropylar cavity, obturator (*obt*), macrospore. Fig. 15, ovule showing integument, obturator, and four macrospores. Fig. 16, macrospore mother cell. Figs. 14, 15, ×250; all others, ×625.

veloped at the two-nucleate stage and remains prominent throughout the further development of the macrogametophyte.

As a result of the nucellar disintegration, the innermost "tapetal"

layer of the integument comes to lie next to the gametophyte. At the four-nucleate stage the cells of this layer appear regularly arranged and have dense cytoplasmic contents.



FIGS. 17–22.—Macrogametophyte development in *Nepeta cataria:* Fig. 17, ovule showing large micropylar cavity, functional chalazal macrospore, and three disintegrating spores. Fig. 18, young ovule containing archesporial cell. Fig. 19, four macrospores. Fig. 20, two-nucleate gametophyte and nucellar layer. Fig. 21, egg apparatus and polar nuclei. Fig. 22, egg (*e*), one synergid, polar nuclei, disintegrating antipodals, nucellar cells, and "tapetum." Fig. 17, ×250; all others, ×625.

Monarda didyma

Macrospore formation and development of the macrogametophyte proceed as in *M. fistulosa*. The four macrospores are smaller and slenderer than those of *M. fistulosa* (fig. 11). At the two-nucleate stage the macrogametophyte differs in shape from that of *M.*

fistulosa, being broad and not greatly elongated (fig. 12). The size of the mature gametophyte corresponds with that in *M. fistulosa*, but the micropylar portion is less broad (fig. 13).

The integument in *M. didyma* is larger than in *M. fistulosa* and the micropyle is longer. The hypostase is not so conspicuous as in *M. fistulosa*, nor is the obturator so large (fig. 14).

Monarda punctata

Development of the macrogametophyte is essentially as in *M. fistulosa* and *M. didyma* (figs. 15, 16). The integument does not surround the nucellus so early as in *M. fistulosa*, but incloses it by the time the macrospores are formed. The obturator is smaller than that of *M. fistulosa*, about the same size as in *M. didyma*. The hypostase is not so conspicuous as in *M. fistulosa* or in *M. didyma*.

Nepeta cataria

Development of the macrogametophyte proceeds as in the three species of *Monarda* (figs. 17–22). The integument is somewhat massive. When it finally surrounds the nucellus, a large micropylar cavity remains between the nucellus and the integument. No obturator is formed.

Summary

1. Development of the ovule and macrogametophyte are similar, except for minor differences, in *Monarda fistulosa*, *M. didyma*, *M. punctata*, and *Nepeta cataria*. The ovule is anatropous, has a single massive integument, a one-layered nucellus which does not persist, a hypostase tissue, and an integumentary "tapetum."

2. The chalazal macrospore is the functional one and the embryo sac is of the common seven-celled type. *M. fistulosa* has the largest macrospores.

3. An obturator is developed in all but *N. cataria*.

4. The integument incloses the nucellus later in *M. punctata* than in the other species. It is thicker in *M. didyma* and *M. punctata* than in *M. fistulosa* and *N. cataria*.

5. The hypostase is very prominent in *M. fistulosa*, rather prominent in *M. didyma* and *N. cataria*, and not so conspicuous in *M. punctata*.

I am indebted to Dr. C. E. ALLEN for helpful suggestions and kindly criticism throughout the course of the study.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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BRIEFER ARTICLE

TECHNIQUE FOR PREPARING MICROSCOPIC SECTIONS OF WOODY STEMS AND ROOTS

In the autumn of 1934 the writers desired to make microscopic sections of the stems and roots of hard woody desert trees and shrubs. Their method is similar to that used in the preparation of slides of petrifactions. Sections 2–3 inches in diameter, of uniform thickness, and in many cases as thin as 10 μ, were cut. These could be examined with an oil immersion lens and photomicrographs made from them. A brief description of the method follows.

PREPARATION OF SECTIONS.—A section 2–4 mm. thick was cut in the desired plane, the surfaces being parallel. Sections larger than 2 inches were difficult to handle. One surface of the section was then smoothed by first placing coarse sandpaper on a glass plate and rubbing the section on it. After the saw marks were removed, finer grades of sandpaper were used, and the section was finally polished on the finest grade of emery paper. The section so prepared was mounted on a glass slide with Canada balsam. The mounted section was now dressed down to the desired thickness with fine sandpaper and emery paper. The final grinding was done on clean worn emery paper with a very light pressure applied on the strokes. When the section was sufficiently thin, it was removed from the slide with xylene. Following the washing away of grit and balsam with xylene, the section was remounted in fresh balsam of ordinary consistency.

STAINING.—Several different methods of staining were tested. The most satisfactory results were secured by placing the sections, after being cut, in alcoholic stains for a period of a few hours to a day or more, depending upon the hardness of the wood. A few good double-stained slides were made by placing the sections in the counter stain and leaving them there until the stain had had time to penetrate the wood, after which they were dried and dressed down in the manner just described. Some good slides were made by staining with iodine green and orange G. The stain which gave the sharpest differentiation of structures with most woods was made by dissolving 2 gm. of acid fuchsin in 100 cc. of a saturated alcoholic solution of picric acid.

Silver nitrate has certain effects upon woody tissues which may make

it a valuable stain. The following method was used. A section was ground down to about 20 μ in thickness, and then covered with a few drops of 2 per cent solution of silver nitrate. This was allowed to stand two or three minutes and was then washed with distilled water, after which it was exposed to a strong light until it turned brown. It was then air dried and ground to the final thickness and mounted without removal from the slide. In only a few cases were the sections uniformly impregnated with silver, but in nearly all the slides there were areas which showed a particularly sharp differentiation of tissues.

The most satisfactory slides were made without staining. In nearly all of the unstained sections the natural coloring of the wood and the difference in the refractive index of the various structures were sufficient to assure a well defined differentiation of tissues. While this method of sectioning has been found to be laborious, the results have been satisfactory. Sections of soft wood, less than 1 inch in diameter, have been prepared in 20 to 30 minutes, but larger sections of hardwoods have taken as long as four hours.—W. W. Newby and Perry Plummer, *University of Utah.*

CURRENT LITERATURE

The Morphology of Vascular Plants, Lower Groups (Psilophytales to Filicales). By ARTHUR J. EAMES. New York: McGraw-Hill Company, 1936. Pp. 433. Figs. 215 and frontispiece.

The author states in the preface that this book is not intended as a reference work but as a textbook. "The viewpoint is that of broad comparative study with the development of a natural classification and phylogenetic relationships as a goal." The life history and type method of presentation of the older morphological works is discarded and the material for the consideration of morphology from the comparative and phylogenetic standpoints is presented in an order and form more consonant with the ideals of the new morphology. Important new material which has accumulated from recent work, not only on the living but on the fossil lower vascular plants, is included. The descriptive text is concisely arranged under a regular sequence of headings, with the pertinent illustrations conveniently inserted at the appropriate places. These excellent illustrations were all prepared by Mrs. EAMES, and the many original ones she herself drew. They and the original photographs by Mr. W. R. FISHER add greatly to the value of the volume. Each chapter is followed by a brief discussion and summary. There is an excellent 21-page index, so classified and complete as to make comparison of important individual features an easy matter. Such an index makes the book very useful for comparative studies, while the excellence of the print and the almost complete absence of typographical errors add to its attractiveness.

Although the subject matter is treated fundamentally from the phylogenetic standpoint, the sequence in which the orders are considered is not phylogenetic but that found most satisfactory from the teaching standpoint. This leads naturally to the consideration of the living forms first, as an introduction to that of the fossil. The last chapter deals with theoretical views on the organization of the plant body and the discussion of the bearing of the results from living and fossil forms on classification.

The chapters which gave the reviewer most pleasure were X and XI, dealing with the water ferns. The biological features of the two families which are ordinarily included in this group are marshalled in a particularly masterly way and convincing evidence brought forward to show that this is not a natural group, but that the Marsiliaceae have come from Gleichenian stock while the Salviniaceae are more closely related to the Hymenophyllaceae. Then too the proposed classification of the vascular plants (Tracheophyta) into four

groups, the Psilopsida, Lycopsida, Sphenopsida, and Pteropsida, represents a decided step toward a natural grouping, the old classification into Pteridophyta and Spermatophyta having a tendency to obscure the connection of the modern seed plants (gymnosperms and angiosperms) with the ferns. One misses, however, the term Verticillatae in this classification. It would seem more appropriate for the horsetails and their fossil allies than Sphenopsida since it emphasizes the distinctly radial structure not only of the sporophyte but of the gametophyte, and sets this group apart from the others in a very decided way, indicating that they probably have been derived from stock more closely related to the bryophytes than have the other forms of the lower vascular plants. Although this would break up the uniformity of the terminology of the classification, it would seem to be warranted by the distinctiveness of the group. EAMES' view of the origin of the sporangiophore also shows how much importance he attaches to this verticillate character.

There are a few features which the reviewer would have liked to have seen treated differently. These are concerned mainly with embryogeny. D'ARCY THOMPSON has shown that the shape and arrangement of the quadrant and octant cells of the animal embryo conform with the requirements of the laws of minimum surface. That these laws are equally applicable to the corresponding stages of plant embryos, as well as to those of gametophytes, pollen grains, etc., is self-evident. Although EAMES has recognized the impossibility of the application of the old quadrant-organography theory in many cases, he still considers that it holds in some, particularly in leptosporangiate ferns. For example, he states of *Marsilia* (p. 220): "Quadrant segments are formed (Fig. 142E), and these bear definite relation to the first organs; the two outer segments form leaf and root, the inner, stem and foot (Fig. 142F)"; and of *Azolla* (pp. 254–5): "Definite quadrants are formed which develop the four primary organs, leaf, stem, root, and foot, as is typical for leptosporangiate ferns (Fig. 166E)." The first difficulty here is that the quadrants of *Marsilia* and other leptosporangiate ferns may be formed by two longitudinal divisions, instead of one longitudinal and one transverse as postulated by the quadrant theory. In this case each quadrant cell would represent parts of two organs, for example, part of the root and part of the foot. Even if it be considered that organ segregation is delayed until the octant stage, a complex change in the mechanism of organ segregation would be involved, and its postulation would be of slight avail since at a later stage the cells from one sector reinforce those from another in forming the first adult organs. The problem is certainly a much more complicated one than the quadrant-organography theory can explain. A further difficulty has to do with one of the postulated four quadrant organs, the so-called foot. The embryo in its early stages absorbs food through all its peripheral cells. Later a specialized area of contact for absorption (an haustorial region) may be formed. This is not necessarily located at the base of the archegonial axis as in bryophytes,

so that the term foot, which implies such location, is inappropriate. It does occur, however, always in connection with the bulk of the food material of the prothallium, whether it appears on the proembryo (before the adult organs are formed) or on some part of an adult organ, as for example at the base of the first leaf whorl in *Equisetum* or at the base of the first leaves in Marattious ferns. BOWER in his new book, Primitive Land Plants, has fittingly designated such haustoria "opportunist growths"! It certainly should not be given equal rank with true organs as is implied by the quadrant theory. On the other hand EAMES' relegation of the root to the place of an adventitious organ (p. 163, etc.) does not seem to be in keeping with the prominence which he gives it in the quadrant. Much could be said on this point but it would involve a consideration of sporeling organization and its relationship to that of the adult, and this aspect has not been included in the present work. It can only be said that the root physiologically and in origin is the equivalent of the shoot and that their course of evolution is of equal importance. It therefore beclouds the problem to call the primitive type of root in such forms as the Psilotaceae by the term rhizome. It was one of the great contributions of the late Dr. D. H. SCOTT that he recognized this and by the use of inverted commas around his terms "root" and "shoot" provided botanists with means of readily designating the primitive type of subterranean and subaerial organs of the plant. There is another aspect of embryogeny to which it seems appropriate to refer here. EAMES, in striking contrast to BOWER, regards the suspensor as of "little or no morphological significance" (p. 297) and even discounts its physiological function. While not attempting to reconcile these opposing views, certain important features may be pointed out. Depth of location of the cells from which the embryo proper develops permits an advanced formation of its adult organs while still within the prothallium, thus allowing the plant to get a good start in life. The original depth of the egg is thus important and the deepening of embryogeny by a suspensor equally so, whether its cells actively elongate and "push" or are passive.

There is one disadvantage which is inherent in the very nature of the textbook type of presentation: the evidence for the opinions expressed cannot always be included and the reader is at a loss as to the basis upon which they are founded. It would be gratifying to know upon what evidence the statment on p. 351 that heterospory "had led to the seed habit" is founded. There are also some statements on the character of the tapetum which it would be interesting to be able to trace to their origin.

The foregoing are minor criticisms, however, in view of the general excellence of the book and the value it will have in revivifying interest in an important field which has been neglected in recent years because of lack of a suitable text.—R. B. THOMSON.

Quantity of Living Plant Materials in Prairie Soils in Relation to Runoff and Soil Erosion. By J. E. WEAVER and G. W. HARMON. Bull. 8, Conservation Dept. of Conservation and Survey Division, Univ. of Nebraska. 1935.

Comparison of Runoff and Erosion in Prairie, Pasture, and Cultivated Land. By J. E. WEAVER and WM. C. NOLL. Bull. 11, Conservation Dept. of Conservation and Survey Division, Univ. of Nebraska. 1935.

Relative Efficiency of Roots and Tops of Plants in Protecting the Soil from Erosion. By JOSEPH KRAMER and J. E. WEAVER. Bull. 12, Conservation Dept. of Conservation and Survey Division, Univ. of Nebraska. 1936.

In view of the importance of the problem of soil erosion and of the recent increase in the activities for combating this national menace, attention should be called to the studies of WEAVER and his students and co-workers. These investigations, carried on at Lincoln, Nebraska, supply needed data of a quantitative nature for certain aspects of the effect of vegetation in impeding soil erosion. Further studies of this type are urgently needed in various parts of the country so that recommendations in regard to soil conservation methods may be placed completely on a basis of fact.

The three bulletins deal with different aspects of the same general theme. In the first the emphasis is upon the consistent decreases in the amounts of underground plant materials found in a series of soil samples taken from relatively undisturbed prairie types, and those secured from the early, medial, and late stages in the deterioration and degeneration of the prairie caused by overgrazing. "On uplands decreases in dry weight were 35, 40, and 72 per cent respectively, from the original sod in the surface 4 inches. . . . . On lowlands decreases of 28, 40, and 77 per cent were found in the surface layer."

The second bulletin reports the results of a study of the amounts of runoff and erosion on prairie, pasture, and cultivated land by the runoff-plot method, in which rainfall was supplemented by artificial watering. "Runoff on a 10° slope from 26.88 inches of rainfall during 15 months was 2.5 per cent from prairie, 9.1 from overgrazed pasture, and 15.1 per cent from a pasture entirely bared by close grazing. The soil was Carrington silt loam. No measurable amount of soil eroded from the prairie, only a small amount from the pasture, but 5.08 tons per acre were lost from the bare area." On April 13, 1935, 2.5 inches of water were applied to these plots at the rate of 2 inches per hour. "Runoff losses were 0, 29.3, and 50.4 per cent, respectively, and losses by erosion 0, 165 lbs., and 3.42 tons of soil per acre. . . . . Runoff on a 5° slope from 12.9 inches of rainfall during a period of 11 months was 1 per cent from prairie, 12.1 per cent from wheat field, and 17.8 per cent from fallow land. . . . . No measurable erosion occurred in prairie, 0.52 ton of soil per acre eroded from the wheat field, and 2.6 tons from the fallow land." Other experiments no less striking bear out the conclusions that "a soil covered with its natural mantle of climax vegetation represents conditions most favorable to maximum absorp-

tion of rainfall and maximum erosion control," that "soils that have been depleted of their organic matter and are poor in structure are less absorptive and are easily eroded," and that "one of the chief essentials of erosion control is the increased use of grasses."

The third bulletin is a detailed report of the relative efficiency of roots and tops acting together and underground parts alone in protecting soil from erosion. Numerous field and garden crops, pasture crops, weeds, and native grasses were tested, and the efficiency was determined by the length of time required for the complete removal of the soil in a sample 1 meter long, 0.5 m. wide, and 1 dm. deep by artificial water erosion under controlled conditions. The samples were secured in the field, so that the soil structure and vegetation were undisturbed. In all cases, as one would expect, the resistance to erosion was much greater when the above-ground plant parts were not removed. In general, the importance of these parts seems to be related to the degree and character of cover by which they prevent the direct impact of falling water upon the bare soil surface. "Prevention of erosion does not result so much from vertical thickness of cover as from one widely spread and continuous." In crop plants "the effect with plant cover intact exceeds that of underground parts alone 3 to 7 times. . . . . Close spacing of rows or broadcasting would result in greatly increased soil protection. Any practice which removes plant cover or débris is undesirable from the viewpoint of soil conservation. . . . . All weeds are of some value as soil conservers. . . . . Remaining on the ground after maturity, they function during life and after death." Space does not allow further introduction of the data or conclusions; anyone seriously interested in the problem of soil erosion should consult these reports.—C. E. OLMSTED.

A Popular Guide to the Higher Fungi (Mushrooms) of New York State. By LOUIS C. C. KRIEGER. New York State Museum Handbook 11, Albany, N.Y.: The University of the State of New York, 1935. Pp. 538 with 126 text figures and 32 colored plates. $2.00.

Few books can compare with this handbook in vivid expression combined with scientific accuracy and high quality of illustrations. It is a responsible presentation by one who is an expert, not alone in faithfully delineating form and color, but in presenting each illustration as a composition. Chapters include an instructive account of fairy rings with a list of the species commonly forming them; a discussion of the seasonal occurrence of certain genera and species, with a tabular summary; habitat relations of important species, particularly with regard to New York State, ably summarized. There is a chapter on the collection and preparation of mushrooms for the herbarium that should be valuable for the beginner and also afford a standard for the serious student of mycology. Another chapter contains a number of recipes for preparing mushrooms for the table—desiderata not to be ignored in a book of this character. A chapter on

their cultivation is a condensed description of usual practice. A discussion of the poisonous mushrooms of the state deals with the more dangerous species and ready means for distinguishing them, symptoms produced by them and the requisite treatments. A chapter on wood-destroying fungi, although containing much interesting information, may seem somewhat extraneous, even though some of the species are mushrooms. The literature of the subject includes extensive citations arranged according to genera. Then follows the systematic account, over half of the book, containing a chart showing the classification of important genera accompanied by pertinent line drawings, a workable key to orders, families, and genera, a discussion of taxonomic characters and their terminology, followed by specific descriptions. These latter employ the least possible technical verbiage without loss of accuracy. It must be borne in mind that throughout there are references to some 600 additional related species, so that the work is actually more comprehensive than is indicated by the 213 species especially treated.

Completing the book are an excellent bibliography, a carefully selected glossary of technical terms, a set of 32 colored plates, and a well elaborated index. There are few typographical errors and the volume is a credit to the institution sponsoring it. It is a landmark among publications on fungi, and will be useful both to serious students and to those whose interest is chiefly gastronomic.

It is unfortunate that at this time another printing of this work, at a higher price and ostensibly covering a greater field but differing only in some title modifications and a few deletions, including the omission of a map, has been issued by a commercial concern and credited to the same author, although apparently without his knowledge. A general handbook of fleshy fungi for a larger area would properly have included a much greater number of KRIEGER'S beautifully executed paintings of mushrooms which have not yet been published and which are not available to the general public.—W. W. DIEHL.

Lehrbuch der Botanik für Hochschulen, begründet von Strasburger, E.; Noll, F., Schenck, H., und Schimper, A. F. W. By FITTING, HANS; HARDER, RICHARD; SIERP, HERMANN; KARSTEN, GEORGE. Jena: Gustav Fischer, 1936. Pp. xii+628. figs. 868.

For many years STRASBURGER had been planning the comprehensive textbook of botany which made its first appearance in 1894. At that time, Drs. NOLL, SCHENCK, and SCHIMPER were associated with him in the famous laboratory at Bonn. STRASBURGER wrote the morphology; NOLL, the physiology; SCHENCK, the bryophytes and pteridophytes; and SCHIMPER, the spermatophytes. The plan and scope of each part was discussed before the writing; and afterward, by repeated conferences, duplication and overlapping were eliminated. That the plan was good and the work well done are proved by the fact that, in spite of changing authorship, this is the nineteenth edition. There were

five editions before the death of SCHIMPER; STRASBURGER wrote eleven editions; NOLL, nine; and SCHENCK, sixteen. Consequently the fundamental ideas of the book were thoroughly established. KARSTEN, who was also associated with STRASBURGER in the Bonn laboratory, took SCHIMPER'S place and has written thirteen editions. In the physiology, NOLL wrote nine editions; JOST, six; while for SIERP, this is the second and for HARDER, who followed SCHENCK, it is also the second.

The new book is a thorough revision, with most of the former material retained but with differences in viewpoint. HARDER recognizes alternation of generations in *Cladophora*, *Ulva*, and *Enteromorpha;* but in *Valonia*, where reduction of chromosomes takes place in the formation of gametes, he says one generation is suppressed. In the Phaeophyceae he recognizes an alternation of generations in forms like *Ectocarpus*, *Cutleria*, and the Laminariaceae, but not in the Fucaceae. He reaches the conclusions which one is likely to reach if he assumes that x generation is synonymous with gametophyte and 2x generation with sporophyte. Often they are synonymous, but in cases like *Ulothrix* and *Fucus*, where they are not synonymous, there is confusion. Below the level of sexuality there is only one generation, the x generation. The 2x generation arose by the fusion of gametes; and from this point the history of alternation is to be traced in the evolution of the new 2x generation, and the reduction of the x generation. He finds alternation in the red algae but in many cases does not homologize it with alternation in the greens and browns. Alternation is recognized in fungi like *Puccinia*, although the reviewer would not agree with the limits set for the two generations. In the higher groups, where x generation and gametophyte and 2x generation and sporophyte are synonymous, there is not much difference of opinion, since the old mistaken idea of homologous alternation went into discard.

Aside from some differences of opinion, largely in respect to alternation of generations, the treatment of the thallophytes, bryophytes, and pteridophytes is excellent and brings this subject up to date. HARDER concludes that the origin of the Archegoniates is entirely unknown.

KARSTEN'S part, the seed plants, has not been changed so much in content, the principal changes being in point of view and in the perspective which comes from long and intimate association with the subject. KARSTEN'S earlier researches, especially in thallophytes and gymnosperms, were the beginning of the firsthand knowledge which makes his part of the book so reliable.

To make room for some increase in the amount of material, many of the figures have been made rather small, but they are clear and easily understood. The numerous life history diagrams, with the gametophytic phase in light lines and the sporophyte in coarse lines, while not always in accord with my own theories of alternation of generations, are clear and sum up the subject at a glance. Throughout the work, references are abundant and up to date.—C. J. CHAMBERLAIN.

A. Engler's Syllabus der Pflanzenfamilien. Eine Übersicht über das gesamte Pflanzensystem mit besonderer Berücksichtigung der Medizinal- und Nutzpflanzen nebst einer Übersicht über die Florenreiche und Florengebiete der Erde zum Gebrauch bei Vorlesungen und Studien über spezielle und medizinisch-pharmazeutische Botanik. Elfte ergänzte Auflage. By LUDWIG DIELS. Berlin: Gebrüder Borntraeger, 1936. Pp. xliii+419. Figs. 476.

The eleventh revised edition of ENGLER'S classical Syllabus der Pflanzenfamilien by DIELS brings this standard reference work up to date, twelve years having elapsed since the preceding edition appeared. In size it is almost exactly the same as the ENGLER-GILG edition of 1924, being, however, provided with a few more illustrations. A comparison of the two editions shows changes here and there throughout the text, bringing the work into conformity with developments of the past twelve years. The major groups remain essentially the same, even to the families, although segregates have been recognized in some cases, and some wholly new families are admitted. Perhaps most of the changes have been occasioned by the desire to bring this summary into conformity with the second edition of Die natürlichen Pflanzenfamilien as far as that work has appeared. The advances and changes that have been made are more striking when one compares the clearly printed edition of 1936 (the edition of 1924 suffers badly by comparison) with the original edition of 1892, which consisted of 207 pages without illustrations. In form the eleventh edition closely approximates its predecessors. This work is an indispensable aid to the working botanist, no matter what his field and whether a teacher of the subject or an investigator, as it provides a concise summary of the salient features of the entire plant kingdom following ENGLER'S modification of the EICHLER system.—E. D. MERRILL.